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THE COURSE AND PATHOLOGY OF *TRYPANOSOMA CONGOLENSE* (BRODEN) DISEASE OF CATTLE*

By

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INTRODUCTION

The literature on trypanosomiasis makes surprisingly little reference to Congolense disease of cattle, as such. What studies have been made lack completeness and the pathology, in particular, has been almost ignored. Many workers have attempted to apply to it the results of work done on other trypanosomes and in other animals, large and small. Yet this monomorphic trypanosome is very different from the polymorphic trypanosomes, both in its mode of attack and its response to curative treatment.

The present paper is in the nature of a general experimental survey of the disease, which it is hoped will form a basis for more intensive study of those aspects which may throw light on methods to combat the parasite. It is also hoped to present a clear picture of a syndrome, which has up to the present been confused and difficult of interpretation.

TECHNIQUE

The experimental animals used.—In the selection of the cattle for experiment, no distinction was made between those of the long-horned Ankole type and the Zebu. The calves used were born at the laboratory and were all cross-bred. All animals were kept housed during the period of experiment and given an optimum diet, with plenty of water and mineral supplement.

The strains of T. congolense *used.*—A locally isolated strain, designated the "Kikaya" strain, was used in most of our experiments; latterly the "Buruli" strain, was employed. During the initial work the former strain was kept in cattle; it was latterly transferred to white mice and during the course of numerous passages showed no tendency to vary. When first isolated, its virulence for cattle was of a very high order, adults being killed in two to six weeks; but in the course of time some of its virulence was lost and it caused a more chronic type of disease. The Buruli strain was also, at first, of a high order of virulence; it was used chiefly for reinfection purposes.

Method of infection.—Animals were in all cases infected by the subcutaneous injection of citrated blood. Calves were infected when under two weeks of age.

Blood: physical and chemical studies.—Where not otherwise mentioned, the technique described by Jones (1943) was used, both in the physical

* The work described was carried out in 1940, 1941 and 1942, but could not be prepared for publication until the present year owing to the absence of one of us (R. N. T.-W.-F.) on special duty.

(S.G.L.) and chemical (E.R.J.) determinations. Packed cell volume was determined by centrifuging oxalated blood in duplicate Wintrobe tubes until a constant cell volume was obtained. The buffy layer of leucocytes was not included in the reading. For the differential count, films were stained by Giemsa and 300 leucocytes were classified, using the " battlement " method of MacGregor *et al.* (1940).

Preparation of histological specimens.—Tissues were fixed in 5 per cent. formol saline and stained with Mayer's acid haemalum and eosin, duplicate slides being stained with Van Gieson. In the case of pancreas, fixation was tried with Zenker's fluid, Zenker without acetic acid and with Helly's fluid ; the latter two gave better results, and our experience was, therefore, similar to that of Dunn *et al.* (1943). Fixation of warm living pancreas was carried out immediately after death in the case of animals destroyed *in extremis*, and in any case within a few hours of death.

THE COURSE OF INFECTION

The trypanosome appeared in the peripheral blood 5 to 19 days following infection. After 13 to 25 days, animals showed a fluctuating temperature, usually higher in calves than in adults. This period probably corresponds to the " trypanolytic " crisis, with antibody formation, as described by Ritz (1914, 1916) and Mutermilch and Salamon (1928). Death sometimes occurred at this stage, usually preceded by recumbency of 24 to 72 hours. Where death did not occur, the animal became subject to a chronic, wasting disease, with severe anaemia and fever of a relapsing nature, to which calves were more resistant than adult cattle.

This fever might continue for five to seven months, after which adult cattle died and calves recovered. Death might, however, occur at any of the relapse periods, which could precipitate a further crisis. At the 8th to 12th week of infection a prolonged crisis period developed, which we call, to differentiate it from the trypanolytic crises, the " anaemic " crisis ; it appears to represent the stage described by French and Hornby (1935, 1936) as occurring within three or four months. Anaemia up to this point was progressive and a low point of resistance was reached ; in animals that survived, the anaemia remained more or less stationary in adults, but calves began to recover.

Recovered cases did not reveal the presence of trypanosomes in blood smears or on sub-inoculation of susceptible animals ; it therefore appears that auto-sterilisation is complete. A second injection with the same strain of trypanosome sometimes produced a transitory reinfection, which failed to develop a third time. The state of " premunity " described by Parkin (1935) as " . . . a state of tolerance of the parasite with a high red cell count," could not therefore be demonstrated, and our findings thus differ from both French and Hornby (1935, 1936) and Parkin (1935).

Table I shows the course of infection in some of our animals.

TABLE I

TABLE SHOWING COURSE OF INFECTION WITH *T. congolense*

No.	*A. or C.*	*Inc. Days*	*Duration Days*	*Result of Infection*	*Homologous Reinfection*	*Heterologous Reinfection*	*Trypanosomes*
4979	C.	19	116	A. steril.	Infected 7 days	7th day	K.B.*
4983	C.	8	177	„	„ 6 „	„	K.B.
4994	C.	11	195	„	—	„	K.B.
4995	C.	9	137	„	—	„	K.B.
4996	C.	10	182	„	—	„	K.B.
4971	C.	7	219	„	Infected 1 day	„	K.B.
4972	C.	9	77	Death	—	—	K B.
4997	C.	11	93	„	—	—	K.B.
5034	C.	5	30	„	—	—	K M.
5035	C.	6	58	„	—	—	K M.
4221	A.	6	4	„	—	—	K.B.
4441	A	6	82	„	—	—	K.B.
4443	A.	6	27	„	—	—	K.B.
4471	A.	6	33	„	—	—	K.B.
4412	A.	5	72	„	Both died shortly after treatment with Stibosan		K.B. & B.B.†
4261	A.	5	73	„			K.B. & B.B.

A. or C. = Adult or calf. K.B. = Bovine Kikaya strain.
D = Died. K.M. = Mouse passaged Kikaya strain.
A. steril. = Auto-sterilisation. B.B. = Bovine Buruli strain.
* Serum protected 5/5 mice against infection with Kikaya strain *T. congolense.*
† „ 2/5 „ „ „ „

THE SYMPTOMS

The symptoms noted do not differ markedly from those described by Hornby (1921), French and Hornby (1935, 1936), and Parkin (1935). We differ from Parkin in finding the disease less severe in very young animals.

Calves always had a bright appearance and their condition only became poor during stages of crisis; the coat was then harsh and staring and the appetite lost. After the crises appetite was regained and the appearance restored to something surprisingly like normal. In spite of this, the anaemia was severe, and persisted for a long period. Weight increase was less than in normal calves and recovery was slow. The general trend of the chronic disease in calves was towards recovery.

In adult animals, in contrast to calves, the trend of the disease was towards death. The picture was therefore different; during the initial crisis period the temperature reaction was far less severe; appetite was lost but recovered when the crisis was past; the coat became harsh and staring at the critical period; and the emaciation and anaemia were never thrown off until death occurred.

Discussion of Course and Symptoms

The justification for describing the fever as relapsing in type is seen in the accompanying temperature chart (Fig. 1). It has been

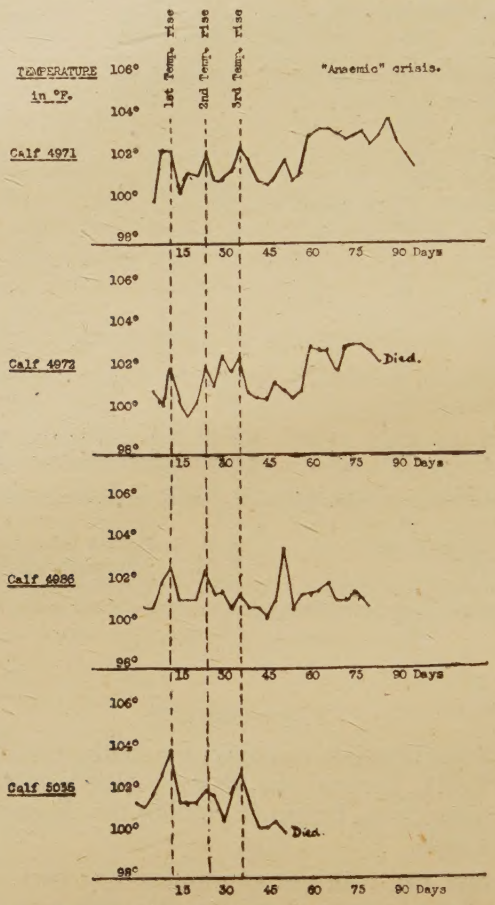

FIG. 1.—Charts showing "smoothed" temperature curves of calves infected with *T. congolense*.

N.B.—The initial temperature rise has in each case been plotted so as to fall on the same day.

necessary in these to plot a three-day average in order to "smooth" the curve, otherwise the daily variations, which occur even in normal cattle, tend to obscure the result. The duration of fever is shown to have been usually one to two days and the interval was in most cases 12 days. The periodicity is not so clear cut as in the relapsing fevers of Spironemal origin, and at times fever became continuous with periods of greater severity; nevertheless, the general trend is clearly shown. The daily counts of the trypanosomes in capillary and venous blood showed such variations, probably owing to weather

and other changes of environment, that it was impossible to determine any correlation between crisis and trypanosome numbers. No attempt has so far been made to ascertain variations of antibody titre of the blood. In claiming that the fever is of the relapsing type, therefore, we rely solely on the temperature curve.

THE PHYSICAL CHANGES OF THE BLOOD

Red cell count.—From the date of the initial crisis the red cell count fell rapidly until about the 12th week of infection. In adult cattle deaths occurred during this period and in those surviving, the count remained at a low level until death some weeks later. In infected calves, however, only two deaths occurred during the "anaemic" crisis, and those surviving gradually improved until, at about the 26th week, the count had approached the normal for that age (Fig. 2).

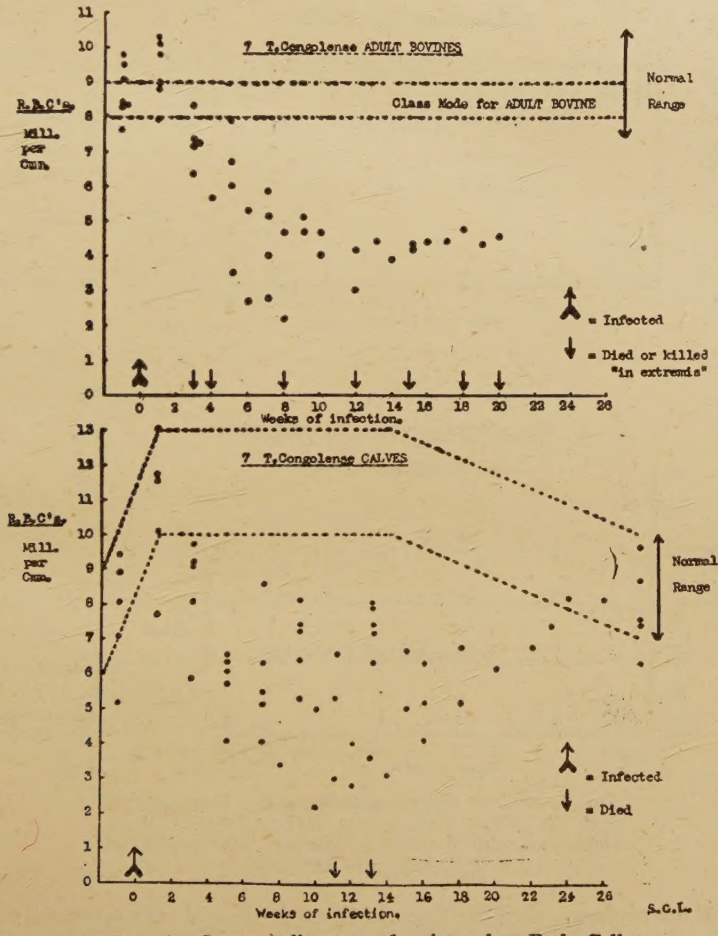

FIG. 2.—Scatter diagram showing the Red Cell count in adult bovines and calves during the course of *T. congolense* infection.

Packed cell volume.—As the anaemia progressed the packed cell volume was reduced from the normal* (40 to 45 per cent.) to a figure as low as 11 per cent. in the adult cattle and 13 per cent. in calves. The Mean Corpuscular Volume was lower than the normal during the first weeks of infection but then tended to rise to the upper limits of the normal range* (35 to 45 c.*).

Haemoglobin.—The haemoglobin was reduced in a somewhat similar manner to the red cell count. The Mean Corpuscular Haemoglobin tended to rise at the anaemic crisis above the normal range* (12 to 15 yy), remaining high in adult cattle until death, but soon reverting to the normal range in calves. The Mean Corpuscular Haemoglobin Concentration in adults was higher than the normal range* (30 to 35 per cent.), but was not outside this range in calves (Fig. 3).

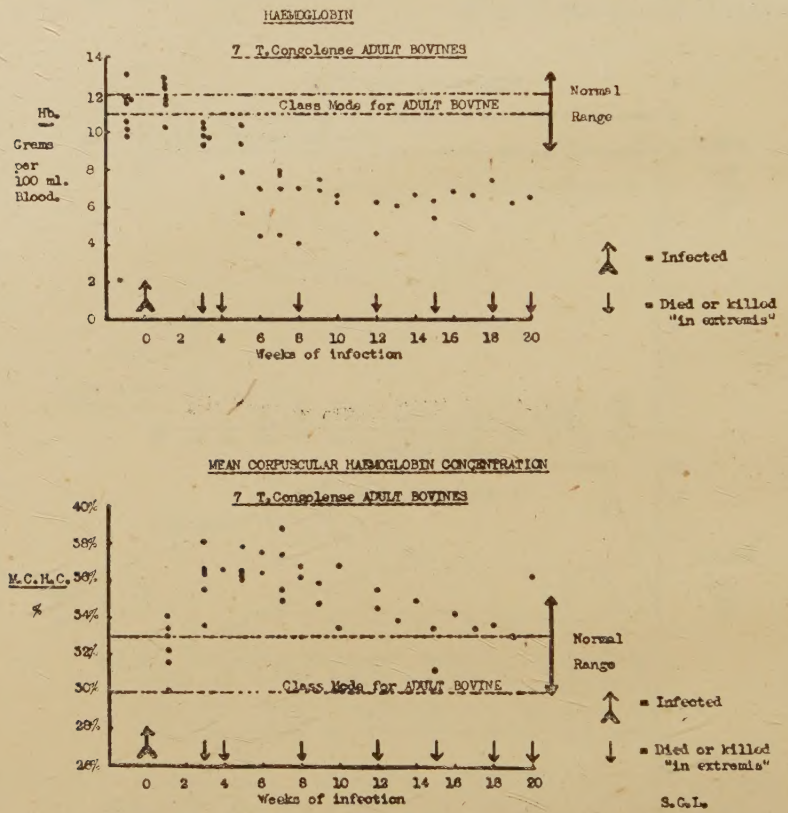

FIG. 3.—Scatter diagrams showing the Haemoglobin and the Mean Corpuscular Haemoglobin Concentration in adult bovines during the course of *T. congolense* infection.

Cell morphology.—In films from adult cattle the red cells appeared normal in size and staining reaction. Microcytes and megalocytes were occasionally

* Unpublished data.

present but anisocytosis was never marked. In some of the calves, punctate basophilia, polychromasia and a slight degree of anisocytosis were present during the anaemic crisis; normoblasts were present, but never in large numbers.

Leucocytes.—In adults there was a gradual decrease in the leucocyte count, the lowest figure being reached at the period of anaemic crisis. This was due to a deficiency of neutrophils, the condition being a pure neutropenia. In calves the leucocyte count reached the high level of 13,000 to 17,000 per c.mm., at the period of anaemic crisis. This was due to a relative and absolute increase of lymphocytes, the other leucocytes remained within the normal range, which varies widely during the growth period (Fig. 4.)

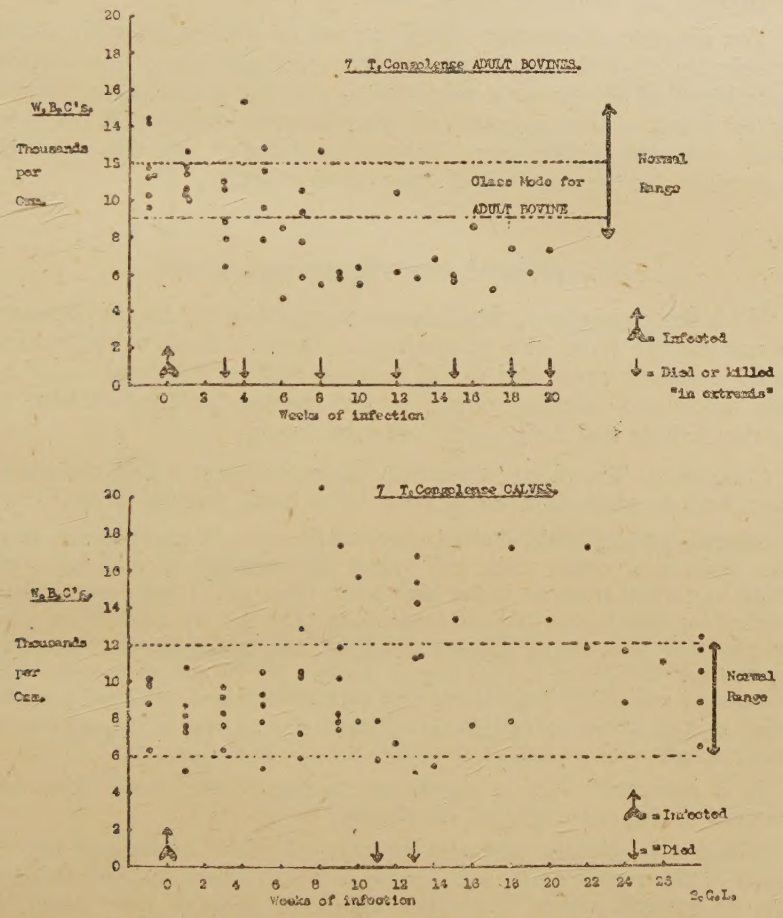

FIG. 4.—Scatter diagrams showing the leucocyte count in adult bovines and calves during the course of *T. congolense* infection.

Discussion of the Blood Physical Findings

The anaemia appears clinically to be non-haemolytic, which is supported by a limited number of icterus index determinations on

the calves, which all gave figures within the normal range. No serum bilirubin estimations have been made. The anaemia was neutrocytic, but with a hyperchromic tendency, without megaloblastic reaction or definite anisocytosis. Sections of bone marrow from two cases have been examined; the great excess of normoblasts in one of them, coupled with their rarity in the circulating blood, supports the hypothesis that the anaemia is of the simple fever type, as described by Saifi and Vaughan (1944). Nevertheless, the aplastic nature of both these bone marrows suggests that there was also some other complicating factor, possibly connected with the thyroid deficiency described later.

The absence of neutropenia in calves, as compared with adults, is probably of little significance, since the number of neutrophils in the blood of the growing animal is so variable as to mask a possible low tendency. The increase of lymphocytes in the calf, as compared with the adult, may indicate a defence reaction partially responsible for the survival of the younger beast.

THE CHEMICAL CHANGES OF THE BLOOD

With few exceptions, the changes recorded in the blood chemistry do not depart from the physiological range. Results for various constituents are presented in the form of scatter diagrams, in which class mode and working range, as determined by Jones (1943) for healthy Ankole and Zebu stock, are also shown.

Cholesterol.—The blood cholesterol changes followed closely the red cell count, both in adults and calves, lower levels being reached in the former.

Glucose.—Adult cattle showed some tendency to hypoglycaemia at the time of anaemic crisis, while calf values followed the normal age curve, falling to normal from an initial high level (Fig. 5) .There was a relative terminal increase in one calf and four adults (Fig. 6). In ox 4443, in which the pancreas was totally destroyed by fat necrosis, glucose was almost absent from the blood on the day of death. As all our cases were killed *in extremis*, except ox 4443 above and calf 4997 (blood collected two days before death), it is possible that the ultimate disturbance of glucose had not been reached.

It is concluded that no significant changes of the blood sugar were observed during the course of the disease.

Non-protein nitrogen and urea nitrogen.—In four adults and one calf values in the region of 40 mg. per cent. N.P.N. were reached about the time of anaemic crisis. No other significant changes were noted, but insufficient data have been collected to make a more definite statement.

Uric acid, creatinine and amino nitrogen.—There were no significant changes in either adults or calves.

Total protein.—The few observations made showed low values in both adults and calves at the time of anaemic crisis.

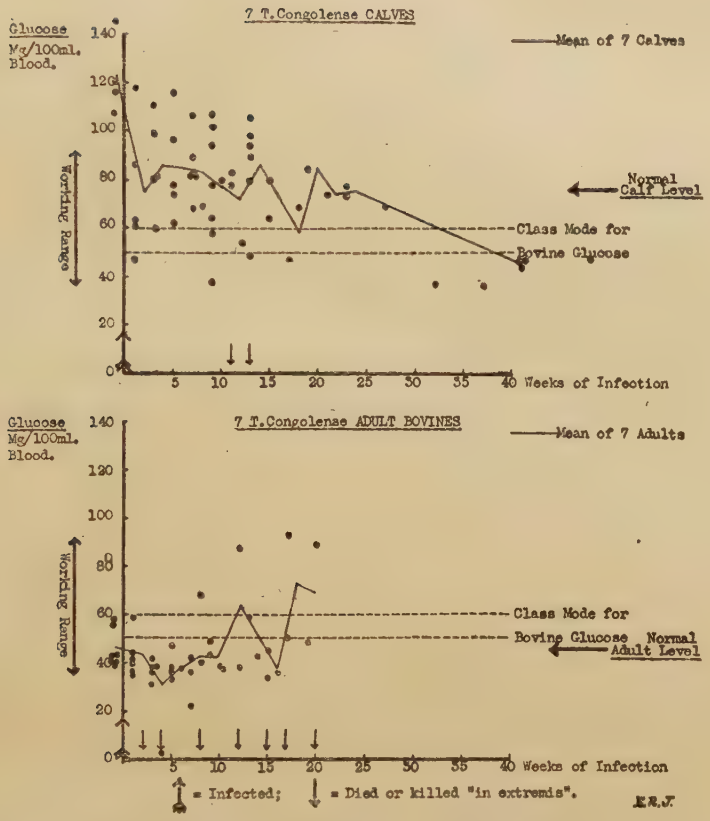

FIG. 5.—Scatter diagrams showing the course of blood glucose in calves and adult bovines during *T. congolense* infection.

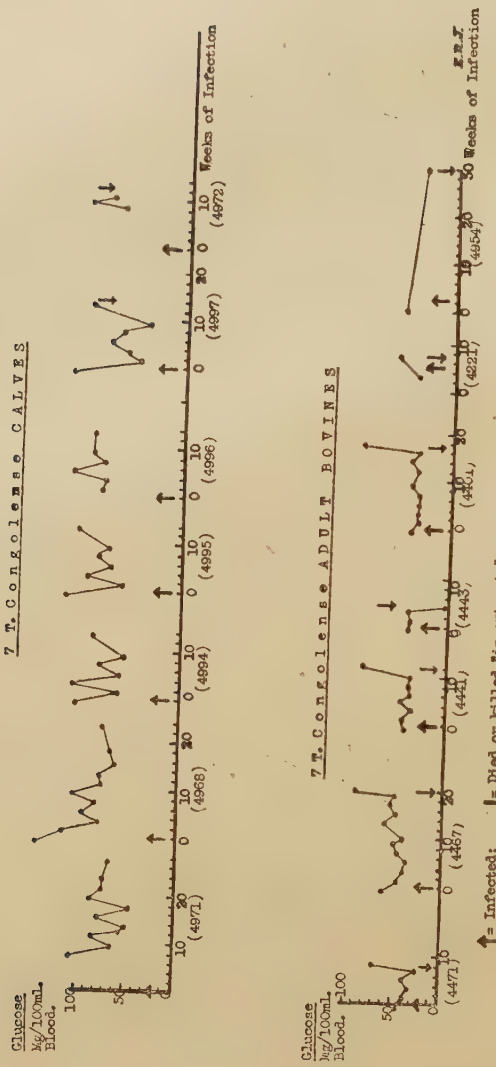

FIG. 6.—Graphs showing the individual course of blood glucose in calves and adult bovines during *T. congolense* infection.

Chlorides.—The blood chlorides were increased at the time of anaemic crisis to approximately the same level in both adults and calves. In calves the level gradually dropped to the upper limit of the normal working range, but in adults it tended to remain high. The maximum increase appeared in calves from the sixth, and in adults from the eighth to tenth, weeks of infection (Fig. 7).

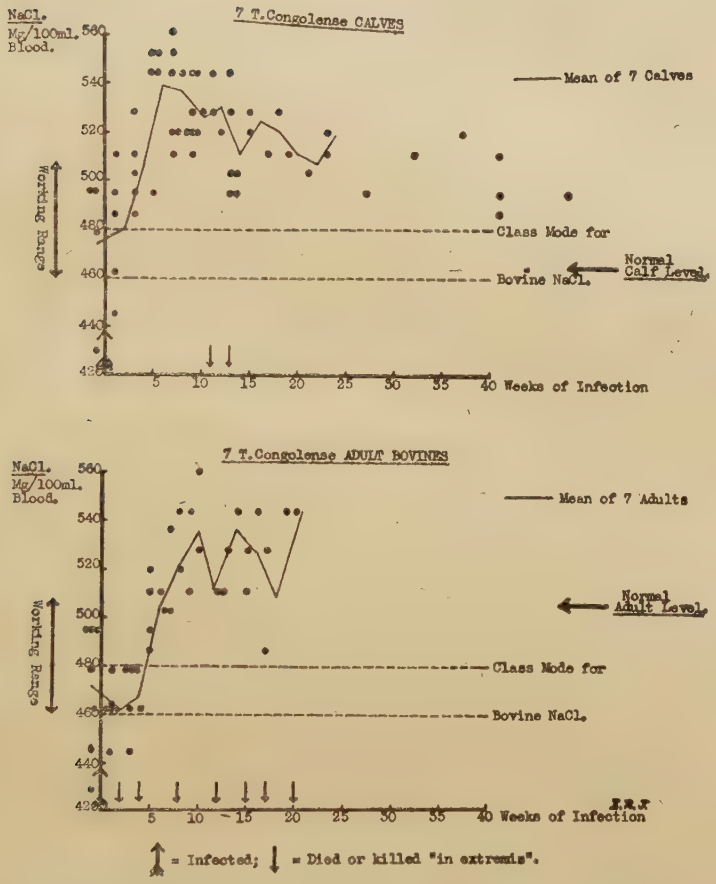

FIG. 7.—Scatter diagrams showing the course of blood chlorides in calves and adult bovines during *T. congolense* infection.

Calcium.—Adults showed a tendency to low levels at the time of anaemic crisis; otherwise it remained normal in both calves and adults (Fig. 8).

Inorganic phosphorus.—In adult cattle the level was gradually decreased, but in calves it remained within the normal range (Fig. 9).

Magnesium.—Both adults and calves showed a slight decrease during the time of anaemic crisis, which slowly returned to normal (Fig. 10).

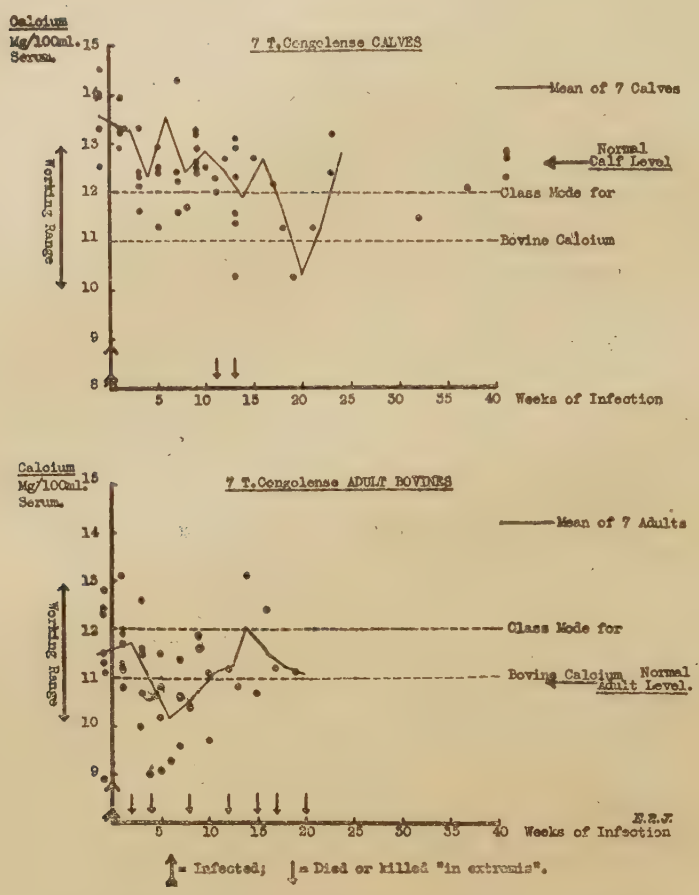

FIG. 8.—Scatter diagrams showing the course of serum calcium in calves and adult bovines during *T. congolense* infection.

Discussion of the Blood Chemical Findings

In assessing our results we do not apply the term hypoglycaemia to glucose values above 40 mg. per cent., as estimated by the original method of Folin and Wu, generally admitted to give somewhat low figures below 60 mg. per cent. Different techniques may account, to some extent, for the varying records given by other workers. Thus French (1938*a*) considers values in the region of 46 mg. per cent. as indicating terminal hypoglycaemia, whereas Daubney and Hudson (1941) find this figure a normal in some healthy cattle. Hudson (1944) regards figures below 20 mg. per cent. as indicating severe hypoglycaemia, but does not apply the term to a 46 mg. per cent. value. Varying results have been recorded also at this laboratory; Jones (1943) reported a mean of 63·3 mg. per cent. for healthy

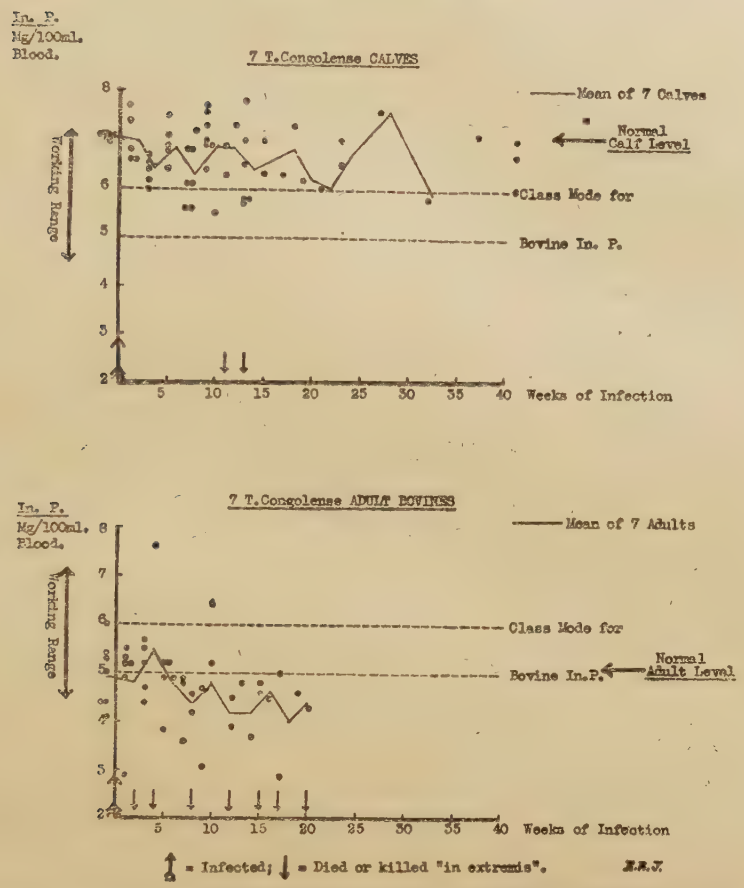

FIG. 9.—Scatter diagrams showing the course of blood phosphorus in calves and adult bovines during *T. congolense* infection.

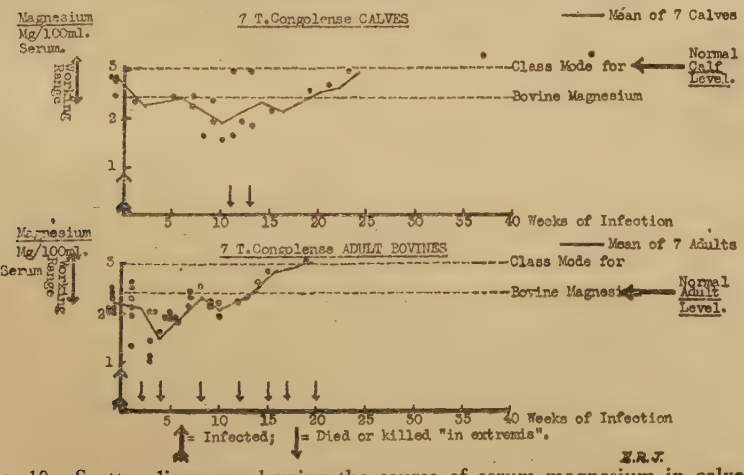

FIG. 10.—Scatter diagrams showing the course of serum magnesium in calves and adult bovines during *T. congolense* infection.

Ankole and Zebu stock, and Bell and Jones (1945), working on glucose tolerance, found a mean figure of 53·3 mg. per cent. in healthy cross-bred Ankole-Zebu adults, but one with a mean blood sugar as low as 46·1 mg. per cent. Interpretation of small changes is therefore difficult, and it cannot, in consequence, be claimed that our findings show any significant change, though the slight hyperglycaemia demonstrated to occur before death is generally at variance with the findings of other workers. Hudson (1944), however, also recorded a high glucose figure in one of his *T. vivax* cases on the day of death.

The high N.P.N. values confirm the results of other workers, but their comparative infrequency, together with the absence of any significant increase in the amino-N., suggest that there is no serious liver dysfunction.

The increased blood chlorides confirm the findings of French (1938*b*).

The calcium findings also confirm the results of French (1938*b*), though possibly our low values may be of more significance than his, in view of our higher control figures. The decrease of inorganic phosphorus corresponds with that reported by Hudson (1944) in mixed trypanosome infections. No previous reference to the serum magnesium level in trypanosomiasis can be traced. The figures obtained for these constituents appear of little significance in themselves, but it is noteworthy that in adult cattle, not in calves, the Ca : P ratio is severely upset (Fig. 11). Whether this variation between the findings in adults and calves is of any significance as regards the death of the one and recovery of the other, cannot be stated; it may merely be a pointer to some other lesion more severe in the adult, which is of lethal significance. Henderson *et al.* (1931) and Le Roux (1933), as noted by French (1938*b*), have both recorded good results from feeding minerals in the prevention of geophagia in trypanosomiasis cases. It is possible, therefore, that the upset of mineral metabolism may be of more significance than has hitherto been generally recognised.

THE PATHOLOGY

A search of the literature reveals that there has been very little work done on the pathology of Congolense disease, especially the histo-pathology. Balfour (1906) described *post-mortem* findings in cattle and stressed particularly the oedema and the haemorrhagic nature of the lymphatic glands of the neck. Dutton *et al.* (1907 to 1908), Montgomery and Kinghorn (1908 to 1909) and Jowett (1910 to 1911) also described *post-mortem* findings, which correspond reasonably well with our own. Of the textbooks, no information is given by Laveran and Mesnil (1912), Knuth and du Toit (1921) or Kolle, Kraus and Uhlenhuth (1929). Hornby (1921) quoted the paper of Balfour (1906) and apart from this he applied to Congolense disease of cattle the findings of Darling (1912) and Simons and

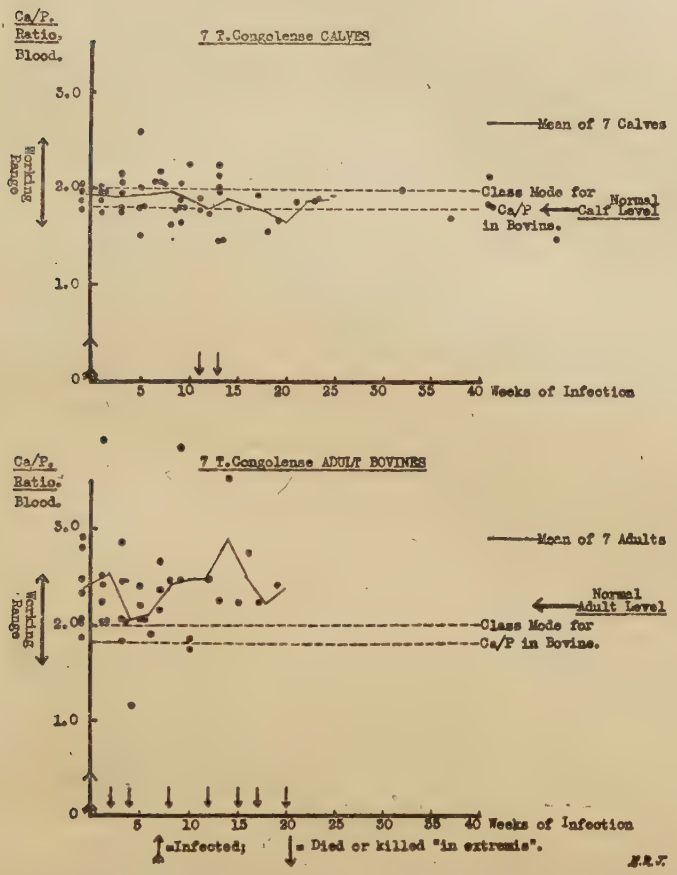
Ca/P. Ratio. Blood.
7 T.Congolense CALVES
3.0
2.0
1.0
0
Working Range
Mean of 7 Calves
Class Mode for Ca/P in Bovine.
Normal Calf Level
5 10 15 20 25 30 35 40 Weeks of Infection
Ca/P. Ratio. Blood.
7 T.Congolense ADULT BOVINES
Mean of 7 Adults
Normal Adult Level
Class Mode for Ca/P in Bovine.
Working Range
5 10 15 20 25 30 35 40 Weeks of Infection
=Infected; = Died or killed "in extremis".

FIG. 11.—Scatter diagrams showing the course of Ca/P ratio in the blood of calves and adult bovines during *T. congolense* infection.

Moenckeberg (1918),who worked with *T. hippicum* in horses. Hoeppli and Regendanz (1930) worked on the histo-pathology of various trypanosome infections of monkeys and dogs ; they concluded that changes seen were dependant on the duration of the infection and not on the species of trypanosome ; this finding, together with the description of the lesions, differs so widely from our own experience that we conclude the work to have no significance so far as Congolense disease is concerned. Schwetz and Fornara (1928) concerned themselves only with the forms of the trypanosome in the vertebrate host and a number of other workers have described various changes in different animals, using different trypanosomes. To some of these reference will be made in the appropriate section. Of systematic work on Congolense disease no reference can be found, though access was not available to the paper of Lavrier (1940) on the cardiac lesion.

TABLE

TABLE OF *post-mortem* CHANGES OF

No.	*Age at Death*	*Duration*	*Condition*	*Abomasum*	*Small Intestine*	*Large Intestine*	*Liver*
5034	2½ months	93 days	Normal	Acute haemorrhagic gastritis	Normal	Normal	Acutely congested swollen
5035	3 „	58 „	Normal	Diffuse inflammation	Muco-enteritis	Normal	Swollen congested
4972	3 „	69 „	Slightly emaciated	Diffuse inflammation	Acute enteritis	Inflammation colon and caecum, ulceration caecum	Congested
4997	4 „	92 „	V. emaciated. Myxoedema	N	N	N	Slightly enlarged
4471	Adult	45 „	Slight emaciation. Myxoedema	N	N	Few ulcers of caecum	Enlarged, gall bladder distended
4441	„	84 „	Emaciated. Myxoedema	Diffuse inflammation	N	N	Enlarged. Cirrhotic (Fluke ?)
4401	„	120 „	Emaciated. Myxoedema	N	N	N	Enlarged
4221	„	19 „	Slight emaciation. Myxoedema	Ulceration	N	Inflamed	Some cirrhosis (Fluke ?)
4443	„	27 „	Slight emaciation. Myxoedema	Diffuse inflammation. Myxoedema of omentum	Enteritis	Enteritis	Enlarged

Gross Pathology

Table II shows the pathological changes in adult cattle and calves. The most noticeable lesion, not altogether constant, was the myxoedematous change of the fatty tissues ; frequently generalised in adults but less frequently in calves, or developed locally in the coronary fat of the heart or the renal fat. Battaglia (1927*a*, *b*) described a similar condition in other trypanosome infections and stressed the myxomatous character of the oedema. The carcass was usually emaciated, but in animals that died in first crisis emaciation was not always markedly advanced.

The liver was usually swollen, congested and dark in colour. Lymphatic glands were affected, either locally or generally, with the cortex fibrosed and the medulla often haemorrhagic. The spleen was sometimes normal in appearance or atrophic with prominent

II

ANIMALS DYING OF *T. congolense* INFECTION

Pancreas	*Lungs*	*Heart*	*Spleen*	*Lymph Glands*	*Kidneys*	*Bladder*
"Fat necrosis"	V. congested	N	Enlarged. Corpuscles prominent	Pale and fibrous	Dark and congested	—
Pink firm	Emphysema of apical lobe	Myxoedema of coronary fat	N	—	N	—
—	Congested oedematous	Epicardial haemorrhages	N	Enlarged oedematous	Congested	Cystitis
—	Congested	Myxoedema, coronary fat	Atrophic	Oedematous	Myxoedema, renal fat	—
"Fat necrosis"	Emphysema, left apical lobe	Pericardial and endocardial haemorrhages. Myxoedema coronary fat	Enlarged	Oedematous	Pale. Myxoedema of renal fat	—
—	N	Pale and muscle flabby	Atrophic	Cortex fibrous, medulla pale	Fatty, yellow	—
—	Atrophic collapsed	Atrophic	N	Cortex fibrous	Fatty	—
—	N	Inflammation of endocardium. Fluid pericardium	Enlarged haemorrhagic	—	Congested. Myxoedema of renal fat	—
—	Oedematous	Pericarditis and endocarditis. Blood stained fluid in pericardium. Myxoedema of coronary fat	Enlarged	TB. of prescapular gland	Much enlarged	—

Malpighian corpuscles or in cases of death at first crisis enlarged. The kidneys always showed signs of necrosis. The lungs often showed areas of emphysema, especially in the apical lobes, more rarely over the whole surface. The heart was often normal, but the muscle was in some cases flabby and necrotic with sub-epicardial or sub-endocardial haemorrhages or both. Often there was fluid in the pericardial sac, which might be blood tinged, and in the thorax and peritoneum. The red marrow extended throughout the length of the long bones in both calves and adults. The blood was thin and watery and the heart usually empty. Gastritis and enteritis were a constant feature.

The general impression was of an emaciated bovine carcass, in which there was a myxoedematous change of fatty tissue.

TABLE

HISTO-PATHOLOGY OF ANIMALS

No.	*Lymph Gland*	*Pancreas*	*Liver*	*Spleen*	*Heart*
4471	Some fibrosis of medulla	"Fat necrosis"	Peri-portal round cell infiltration, sinusoidal ditto	N	Degeneration of fibres
4937	Hyperplasia conn. tissue especially medulla. Some B.V.s thrombosed	Necrosis islet tissue	Periportal and some sinusoidal R.C. infiltration	N	Some fibres degenerate
4441	Hyperplasia conn. tissue medulla	Necrosis islets and slight of parenchyma	As last with fatty degeneration	Petechial haemorrhages. Ring appearance of lymph tissue	N
4954	Slight fibrosis medulla	Necrosis islet and parenchyma	As last	Haemosiderin. Fibrosis especially round B.V.s. Lymph tissue empty of cells	N
5034	Not seriously affected	—	Fatty degeneration. Congestion	—	—
5035	Fibrosis throughout. Haemorrhages medulla	Necrosis islets. Haemorrhages of parenchyma	Periportal and sinusoidal R.C. infiltration. Congested	R.B.C.s numerous	Necrosis of some fibres
4446	—	Some necrosis islets	Fattydegeneration. Periportal R.C. infiltration. Kupffer cells swollen	Ring appearance lymph tissue, which appears increased	Necrosis some fibres
4443	—	—	Fattydegeneration. Round cell periportal infiltration. Congested	N	—
4069	—	—	Cloudy swelling. Periportal R.C. infiltration. Kupffer cells swollen	—	—
4418	Some fibrosis of medulla. Vacuoles in cortex	Slight degeneration of some islets	Some cloudy swelling of parenchyma	Some hypertrophy of trabeculae	N

III

DYING OF *T. congoiense* INFECTION

Kidney	*Adrenal*	*Pituitary*	*Thyroid*	*Lung*	*Bone Marrow*
Necrosis tubular epithelium. Round cell infiltration cortex	Slight sub-capsular infiltration of cortex	Necrosis and fibrosis oxyphil cells in one area	—	—	—
As last	Capsule thickened and fibrous tissue invasion of zona glomerulosa. Fibrous band between fasciculatis and reticularis. Fibrosis of medulla	—	—	—	
As last, but with cystic and consolidated areas	Sub-capsular R.C. infiltration. Capsule thickened at expense of glomerulosa	Colloid cyst anterior lobe R.C. infiltration	—	—	—
As last, but no ystic areas	Glomerulosa extensive degeneration. R.C. infiltration rest gland and medulla	Degeneration oxyphil cells. Some R.C. infiltration	—	—	—
Fatty degeneration. Petechiae. R.C. infiltration throughout	Degeneration cells in cortex and medulla	Haemorrhages and degeneration oxyphil cells widespread	—	Congested. Haemorrhage into alveoli. Areas consolidation	Aplastic. Undue proportion of normoblasts. R.B.C.s fairly numerous
Degeneration tubules. R.C. infiltration round glomeruli. Consolidated areas	As last	—	—	—	—
As last	—	—	—	—	—
Tubular epithelium almost totally destroyed. R.C. infiltration cortex. Fibrosis.	—	—	—	—	—
Tubular epithelium degenerated. R.C. infiltration whole organ and glomeruli. Fibrosis	—	—	—	—	—
Tubular epithelium degenerated. Fibrosis. Perivascular R.C. infiltration	Slight necrosis cortex	Colloid cyst and degeneration oxyphil cells	—	—	—

TABLE III—

No.	*Lymph Gland*	*Pancreas*	*Liver*	*Spleen*	*Heart*
4874	Extensive fibrosis. Numerous clasmatocytes in fibrous bands. Hyperplasia arteriole walls	—	Cloudy swelling. Periportal R.C. infiltration. Kupffer cells swollen	As last. Lymph tissue increased	—
4401	Cortex densely packed with lymphoid tissue. Trabeculae thickened. Clasmatocytes numerous and occlude some B.V.s	Fatty degeneration parenchyma. Islets not seriously involved	Fattydegeneration. Periportal infiltration R.C.s. Kupffer cells swollen	Haemosiderin. Ring appearance lymph nodes	R.C. infiltration between fibres and below endothelium
4971	Fibrosis	Islet tissue necrotic and v. scanty	—	Haemosiderin. Lymph nodes v. diffuse	N

The Histology

Hornby (1929 and 1933) has stressed that *T. congolense* is essentially a blood parasite, a view borne out by the present work. In sections the parasite was never found in islets or nests in the tissues, as in the case of the polymorphic trypanosomes and *T. cruzi*, and there is no evidence that any tissue damage was caused purely by the mechanical action of the parasite itself. Table III shows the tissue changes noted.

The Cellular Reaction.—The general findings in Congolense disease are of a widespread round cell infiltration, which advances to fibrosis. This reaction must be regarded as the primary lesion. Hu (1931) and Hu and Ch'in (1933) studied the cellular reaction, working with *T. brucei* in white rats, and demonstrated by supra-vital staining that it was lymphoblastic in origin ; these findings are similar to our own, and supra-vital staining was also employed to prove the nature of the white cells in the blood. In some sections, however, clasmatocytes are strikingly numerous especially in the lymphatic glands and the possibility of reticulo-endothelial damage must not be overlooked.

Continued

Kidney	*Adrenal*	*Pituitary*	*Thyroid*	*Lung*	*Bone Marrow*
Tubular epithelium degenerated. R.C. infiltration round glomeruli. Fibrosis	—	—	—	—	—
As last	—	Colloid cyst. Oxyphil cells extensively destroyed	—	—	—
Necrosis of epithelium. R.C. infiltration and fibrosis of cortex	Necrosis of glomerulosa but not marked	—	Capsule invaded by clasmatocytes. Hyperplasia of fibrous tissue. R.C. invasion in and around acini. Acini v. full of colloid and some greatly distended; others full of R.C.s, and may be invaded by fibrous tissue. Congestion of B.V.s	—	Aplastic, with a few areas of erythropoietic activity

The lymphatic glands showed a picture of advancing fibrosis. The germ centres were occluded by fibroblasts, which had laid down fibrous tissue, so that they appeared to become isolated. The lymph sinus was blocked by fibrous tissue and the medulla often fibrous and vascular. The germ centres contained a large number of mature lymphocytes, but large and small lymphoblasts were usually more frequent (Fig. 12).

The spleen was either engorged with red blood cells or so empty that the reticular tissue was plainly seen. Occasionally, there were haemosiderin deposits. The lymph nodes often appeared enlarged but fibrosis was not noted in any of our cases and the appearances seen in the lymphatic glands were absent. Often there was a dense ring of lymphocytes towards the periphery of the lymph nodule, which was almost empty of cells at the centre.

The liver showed a varying degree of necrosis of the parenchyma cells. There was a periportal infiltration of cells of lymphoblastic origin which sometimes extended into the sinusoids. The von Küpffer cells often appeared to be swollen. In general, the liver lesion did not appear to be very severe (Figs. 13 and 14).

The kidney lesion was never absent and was almost invariably of a very severe nature. Often almost the whole of the tubular epithelium was destroyed, the course of the tubules being marked by threads of connective tissue, remnants of the basement membrane. Round cell infiltration frequently extended throughout the organ, but was most severe in the cortex and especially around the glomeruli. Areas of extensive fibrosis were commonly seen. The glomeruli were sometimes unaffected, but often showed round cell infiltration and in some cases were stenosed (Fig. 15).

The heart was not always affected ; often the fibres showed some signs of atrophy.

The pituitary was not constantly affected. In three sections there were colloid cysts in the anterior lobe, which appeared to arise from disintegration of the oxyphil cells. Some round cell infiltration and cellular degeneration was seen in the pars anterior, which appeared to attack the oxyphil cells, whose staining and arrangement in trabeculae was lost. Damage was most severe in those parts furthest from the pars intermedia and pars posterior, which were never affected (Figs. 16 and 17).

The thyroid gland. Only three thyroids were collected for examination. They were all extensively fibrosed, one to such an extent as to be too hard to cut into sections with the microtome. It was possible to cut sections from the other two only at the periphery of the gland, the rest also being too fibrous for the knife. The sections showed in the one a great overgrowth of fibrous tissue, with scattered acini and groups of acini in the interstices. In both the acini varied greatly in size and were mostly full of colloid. Invasion by round cells was occurring, not in the cellular structure, but in the colloid of the acini ; they became filled with round cells, shrunken and disappeared into a small fibrous nodule (Fig. 18).

The adrenal was constantly and seriously affected, almost always the cortex and sometimes the medulla. There was round cell infiltration and fibrosis below the capsule and this sometimes extended between the bundles in the zona fascicularis. In one case, there was a complete circle of fibrous tissue between zona glomerulosa and zona fascicularis. The fibres appeared to be introduced at the expense of the cortical cells, which became necrotic and disappeared. Fibrosis sometimes extended into the medulla (Figs. 19 and 20).

The pancreas, in two cases, was totally destroyed by " fat necrosis." Sections showed swollen fatty cells with a few remnants of pancreatic cells in their interstices. In every case examined there has been some damage to the islet tissue. Occasionally, whole islets have disappeared, leaving vacuoles with a few shrivelled islet cells still adherent. The appearances were more like those described by Dunn *et al.* (1943) in cases of alloxan poisoning than of diabetes mellitus in the human being. All cells in the islet appeared to be equally affected, but those at the centre first showed degenerative changes. The nuclei became pyknotic and the cytoplasm eosinophilic ; the central cells often appeared as a mass of cytoplasmic debris, without nuclei (Figs. 21 and 22).

Discussion of Pathological Findings

Aggressive properties of T. congolense.—The most striking difference between the pathology of Congolense disease of cattle and other trypanosomiases lies in the absence of parasites or parasite

forms in the tissues. The parasite nests, which are a feature of *T. gambiense*, *T. rhodesiense* and *T. cruzi* infections, were never found. Nevertheless, the histological findings stand out as the most striking and constant feature of the disease. It is impossible to agree with those workers who consider the anaemia or the alterations in the blood chemistry to be the prime factor ; it appears evident that these factors are secondary to an extensive and serious involvement of certain vital organs, on which responsibility for the maintenance of normal metabolism rests. It would be easy to postulate that *T. congolense* must produce a toxin, by which the tissue damage is wrought ; but the problem cannot be so lightly dismissed. In the first place consideration must be given to the failure of other workers to demonstrate the presence of a toxin, and secondly, the clinical picture does not suggest a toxaemia so severe as to cause such extensive changes. Some other explanation must first be sought and on this it is not possible at present to hazard any conjecture.

The blood chemistry in relation to the pathology.—It is plausible to suppose that the rise in the blood chlorides may bear some relationship to the lesion of the adrenal cortex. Chagas (1916) described bronzing of the skin and other symptoms resembling Addison's disease in children affected with American trypanosomiasis. Though the calves did not appear to have been so seriously affected, a relationship between the blood chloride picture and the cortical gland seems probable.

The severe lesion of the kidney might be expected to lower the renal threshold, and thus contribute to a lowering of the blood sugar, while diseased islet gland and adrenal medulla might be expected to act antagonistically. Perhaps it is not surprising, therefore, that the blood sugar findings described here, like those of other workers, are generally inconstant. Locatelli (1930) described an islet lesion, and Bruynoghe *et al.* (1927) attributed blood sugar changes to insulinic metabolism ; nevertheless, in view of the showing of Mirsky (1942) that insulin does not play the same important part in some animals as in man, caution must be exercised in attaching too much importance to an islet lesion. No confirmation has yet been made of the findings of Bruynoghe *et al.* (1927) that the liver and muscle glycogen is diminished, which might throw some light on the part played by the different influences at work.

The endocrine system.—We have demonstrated lesions, more or less severe, of the anterior pituitary, the thyroid, the adrenal and the islet tissue of the pancreas. Some of these are probably related to the metabolic disturbances, which are a feature of the disease.

Owing to the interrelation of function of the endocrine system, it is difficult to assess which glands are primarily affected and which show changes consequent on a lesion of some other gland. If it is accepted that the primary lesion is a round cell infiltration and fibrosis, then primary lesions may be found in all glands, except the islet ; in the pituitary, however, round cell infiltration is uncommon,

and it is probable that this lesion, too, is usually secondary. We may accept, therefore, that the thyroid and adrenal lesions are primary, and it is not improbable that a pituitary lesion may be secondary to these. The pancreatic lesion could be secondary to a pituitary lesion or, if Dunn *et al.* (1943) are correct in their hypothesis that alloxan is an abnormal product of uric acid metabolism, it might be produced metabolically independently of the endocrine system.

It is thus probable that the endocrine system suffers from primary lesions of the thyroid and adrenal. The former is probably associated with the myxoedema and possibly, as already shown, to some extent with the anaemia. The adrenal may bear some relationship to the high blood chloride figure. It is tempting to draw the analogy of Chagas' disease, in which both these glands are seriously affected, and symptoms of myxoedema, cretinism and resemblance to Addison's disease are a feature. It appears from our studies that Congolense disease is more closely related to this affection than to other trypanosomiases. The original work of Chagas (1916) may with advantage be consulted.

The cause of death.—There has been much controversy regarding the cause of death in animal trypanosomiasis, and many theories have been put forward attributing it to isolated features of the metabolic disturbance. It is not proposed here to review the literature, as in all cases theories have been put forward without a comprehensive study of the disease.

The most significant individual factor appears to be that whereas adult cattle die, calves for the most part recover. It is, therefore, of interest to note what differences there are in the disease as manifested in young and adult animals. In the first place the myxoedema was always far less severe in young animals than in adults ; in calves the Ca : P ratio was normal, whereas adult cattle showed severe disturbance ; again in calves the blood chlorides and the anaemia were gradually restored to normal after the anaemic crisis, whereas in adults the derangement persisted until death. The lymphocytosis seen in calves but not in adults may indicate a greater mobilisation of the cellular elements of resistance. None of these factors is of lethal significance in itself, but they are all, except the lymphocytic reaction, such as might be explained by a less severe involvement in calves of the endocrine system. The thyroid is possibly involved in the myxoedema, the anaemia and in the imbalance of the Ca : P ratio ; the adrenal is possibly involved in the high chloride figures. It is possible, therefore, that some endocrine factor may contribute to the survival of the young animal.

The reasons for regarding the blood changes as secondary to the tissue changes have already been discussed and we look, therefore, for the cause of death among the latter. In the first place, then, it may be suspected that the endocrine system is a contributory cause, leading by its failure to secondary disturbances of metabolism. The severe changes in other organs must not, however, be overlooked.

The primary lesion of round cell infiltration with ensuing fibrosis has been described. In many organs fibrosis appears to extend to arteriole walls and in some sections blockage of venules by clasmatocytes has been noted. Passive congestion of the liver and lungs is a common feature, as is the presence of transudate in the pericardium and thoracic and peritoneal cavities; sometimes there is degeneration of the heart muscle. These findings point to circulatory failure, due possibly to a partial blockage of the smaller blood vessels. If this should be the case, the lethal factor is the cellular and fibrous reaction; on what that is dependent we do not know, though the lymphatic lesion would appear to be of prime importance.

Finally, the kidney lesion is the most constant and the most severe. In it, however, cellular infiltration and fibrosis are confined mostly to the cortex, whereas the greatest degree of cellular degeneration is usually in the medulla. It may be suspected, therefore, that the lesion is a secondary one, due possibly to the passage of toxic products of metabolism in the urine. In the absence of evidence of uraemia, it seems unlikely to be the exciting cause of death.

The appraisement of our results gives little ground for postulating that any one factor is constantly responsible for death in Congolense disease. It is probably of greater interest to study those factors that make for survival of young animals; there is ground for believing that the answer may be found in some variation of involvement of the endocrine system.

The immunity reactions.—A developed immunity to *T. lewisi* and to *T. duttoni* in rats is recognised. In *T. congolense*, Van Saceghem (1934, 1936, 1938) studied it, as did Schilling in numerous publications (1933, 1935*a*, *b*; 1936*a*, *b*, *c*), Schilling *et al.* (1932, 1933, 1934*a*, *b*; 1935, 1936*a*, *b*), Hornby and Bailey (1932) and Hornby (1941), completing the work of Schilling and his collaborators. Dr. Livingstone (Missionary Travels in South Africa) first commented on the resistance of young animals to the disease, and the work quoted above was mostly concentrated on this aspect.

Nevertheless, a true immunity to *T. congolense* is still not recognised, since it is considered that resistance is due to a state of "premunition." It is claimed here that a true immunity exists, as in the case of *T. lewisi*, *T. duttoni* and bacterial infections. The fact that the existence of a multiplicity of strains of the parasite has hitherto defeated the object of producing a fully resistant animal cannot alter this basic fact, when the behaviour of a single strain is considered. The parasite from a single strain failed to alter its aggressive or immunological characteristics after mouse passage. It can evidently produce relapse strains by mutation and probably drug resistant strains, but the one strain remains essentially the same. It is so far typical of the relapsing types of Protozoa.

It must, therefore, be considered whether we are dealing with a multiplicity of strains in the strict sense or with a number of subspecies, which may show on careful examination differences in morphology, physiology and aggressive properties. If a large number

of strains exist in the sense that they do in the streptococci, any attempt to produce a fully immunised animal may well prove hopeless. If there are a number of physiological variants, it is obviously important to find out what they are and what are their differences, with a view to mapping out the geographical distribution of each variant. In future, it is, therefore, important that a careful study of the morphology and measurements of each strain used should be undertaken; the periodicity of the temperature chart is likely to give an indication, where a physiological variant is encountered. Lastly, an attempt may be made to separate strains or variants serologically. In spite of failures recorded hitherto, it is not considered that the time has yet come when this line of research should be abandoned.

SUMMARY

The course and symptoms of Congolense disease in cattle are described.

The disease appears to be a typical relapsing fever, generally leading to death in adult cattle or recovery in calves, with immunity to the homologous but not to heterologous strains of the parasite.

The general features of the anaemia are discussed in detail and classified.

The blood chemistry is studied with special reference to the carbohydrate, nitrogen and mineral metabolism.

The pathology is discussed and related to the symptoms and changes in the physical and chemical blood picture.

It is concluded that the creation of an effective immunity to Congolense disease may not prove impossible.

Note.—It has not, for reasons of space, been possible to publish the figures for each animal. The figures will, however, be made available, on request to this laboratory, to any worker particularly interested.

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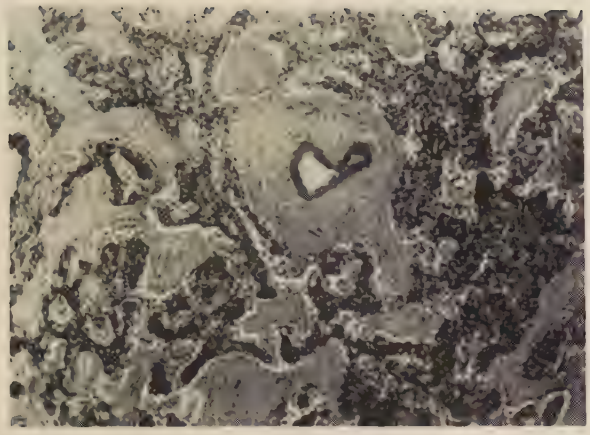


Fig. 12.—No. 4937. Lymphatic Gland, × 200. Medulla. Marked hyperplasia of connective tissue. Haemalum and Eosin.

Fig. 13.—No. 4471. Liver, × 200. Peri-portal round cell infiltration; cloudy swelling and fatty degeneration of liver parenchyma; some congestion. Haemalum and Eosin.

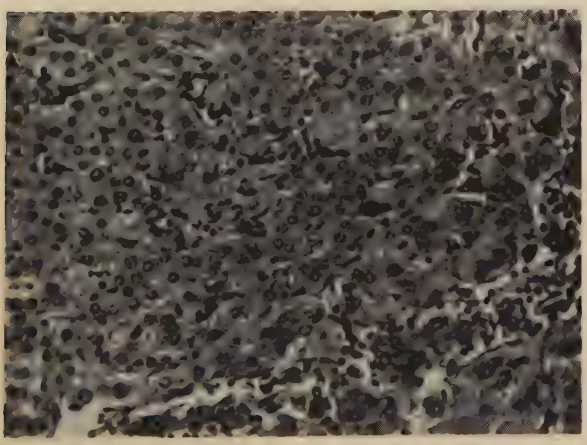


Fig. 14.—No. 4441. Liver, × 400. Periportal and sinusoidal round cell infiltration; swollen von Küpffer cells. Haemalum and Eosin.

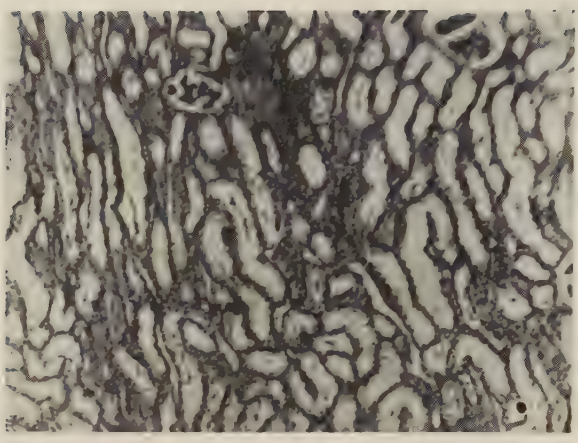


Fig. 15.—No. 4954. Kidney, × 200. Degeneration of tubular epithelium. Round cell infiltration. Sclerotic glomeruli. Haemalum and Eosin.

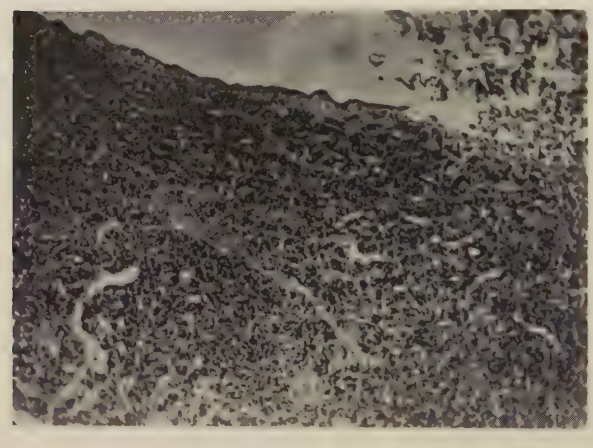


Fig. 16.—No. 4954. Anterior Pituitary, × 200. Degeneration of oxyphil cells. Haemalum and Eosin.

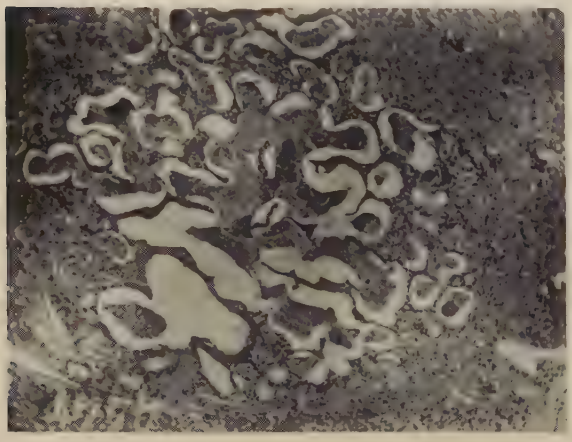


Fig. 17.—No. 4441. Anterior Pituitary, × 200. Large colloid cyst, with degeneration of oxyphil cells Haemalum and Eosin.

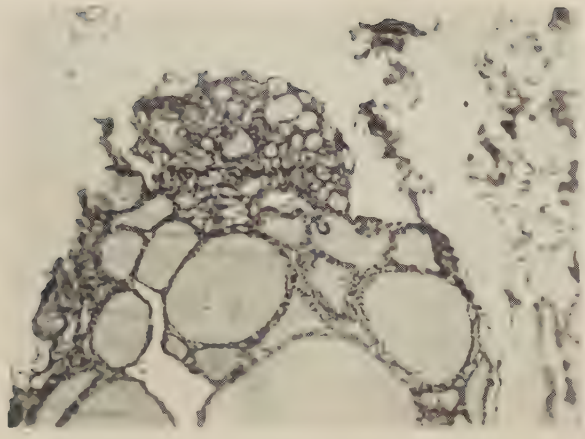


Fig. 18.—No. 5034. Thyroid, × 200. Distension of acini and round cell infiltration leading to atrophy. Haemalum and Eosin.

Fig. 19.—No. 4937. Adrenal, × 200. Fibrous band developed between zona fascicularis and zona reticularis. Haemalum and Eosin.

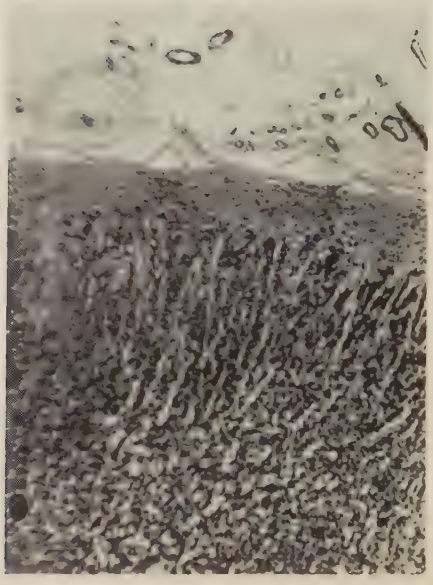


Fig. 20.—No. 4441. Adrenal, × 200. Hyperplasia of fibrous capsule. Round Cell infiltration of zona glomerulosa with degeneration of glandular cells. Haemalum and Eosin.

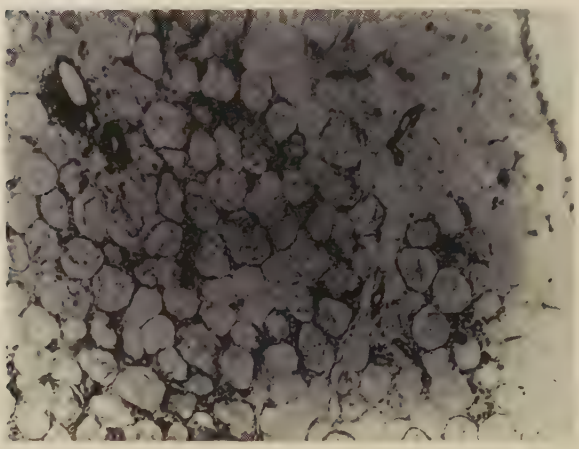
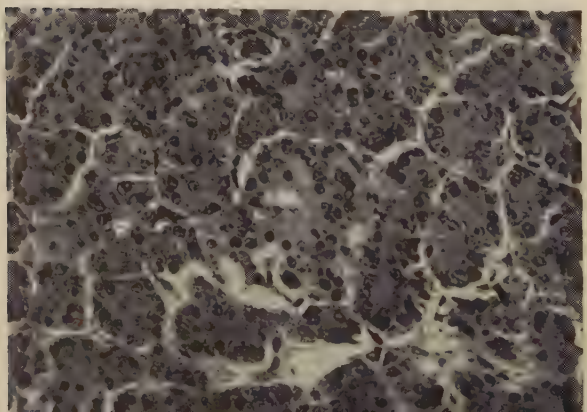


Fig. 21.—No. 4471. Pancreas, × 200. "Fat necrosis" of entire organ. Haemalum and Eosin.



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TRYPANOSOMA CONGOLENSE (BRODEN) DISEASE OF CATTLE: THE PARENCHYMATOUS LESION AND ITS RELATION TO THE CELLULAR DEFENCES

By

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INTRODUCTION

In the preceding communication (Fiennes *et al.*, 1946) the nature of the parenchymatous lesion in *T. congolense* infection was studied and it was possible to ascribe to it the metabolic changes which occur during the course of the disease. The cause of the lesion was not described, though some scepticism on the toxic theory of causation was expressed.

The present study was undertaken to establish the causes which underlay the widespread degeneration of so many essential organs. The study of the cellular defences and the aggressive properties of the trypanosomes is incidental to the main enquiry and has, therefore, been carried only so far as was necessary to prove the underlying source of injury.

THE PATHOLOGY

A careful study of sections from all organs, examined in the previous work, showed that there was multiple thrombosis of very many of the smaller blood vessels, both arterioles and venules. The lesion could adequately be ascribed to multiple infarction, leading to degeneration and replacement fibrosis of the parenchyma. The thrombosis was in most vessels only partial, but all stages between a few red cells adherent to the vessel wall and a fully organised white thrombus could be traced.

The tunica adventitia of most of the vessels was hyperplastic, and this would result in a loss of elasticity and no doubt some diminution in the size of the lumen from contraction. Apart from this, the endothelium was very deficient; the cells gave the appearance of becoming swollen, undergoing metaplasia to a cell of macrophage type and migrating into the blood stream. Careful examination showed that these appearances were not due to agonal stripping. Descriptions of appearances seen in some organs, selected at random, are given in Appendix I and are illustrated photographically in Figs. 5 to 9.

Macrophages were strikingly numerous in all connective tissues, including the walls of the blood vessels, especially the adventitious coat. Some of these were of clasmatocyte type and free from contained granules, some contained eosinophilic granules, which were

large, very numerous, and often spilled out by bursting of the cell, while others contained basophilic granules, which could be traced as the remnants of necrotic tissue debris.

It seemed probable that the eosinophilic granules might be largely ingested trypanosome debris, which proved toxic to the cells and caused them a hard struggle in the reproduction race against the trypanosomes. This would account for the deficiency of endothelium in the blood vessels and might be the underlying cause of the thrombosis. To prove this involved a study of the defence mechanisms against the parasites.

THE BLOOD MONOCYTES

It was previously stated (*loc. cit.*) that the blood monocytes remained within the normal range during the course of the disease. If the theory given above were correct, this could hardly be so. A re-examination of the figures showed that when the numbers of monocytes per c.mm. of blood were plotted against the temperature curve there was a tendency to fall at the time of the crisis and a distinct rise, when this was over (Fig. 1). This indicated the possibility of macrophage involvement.

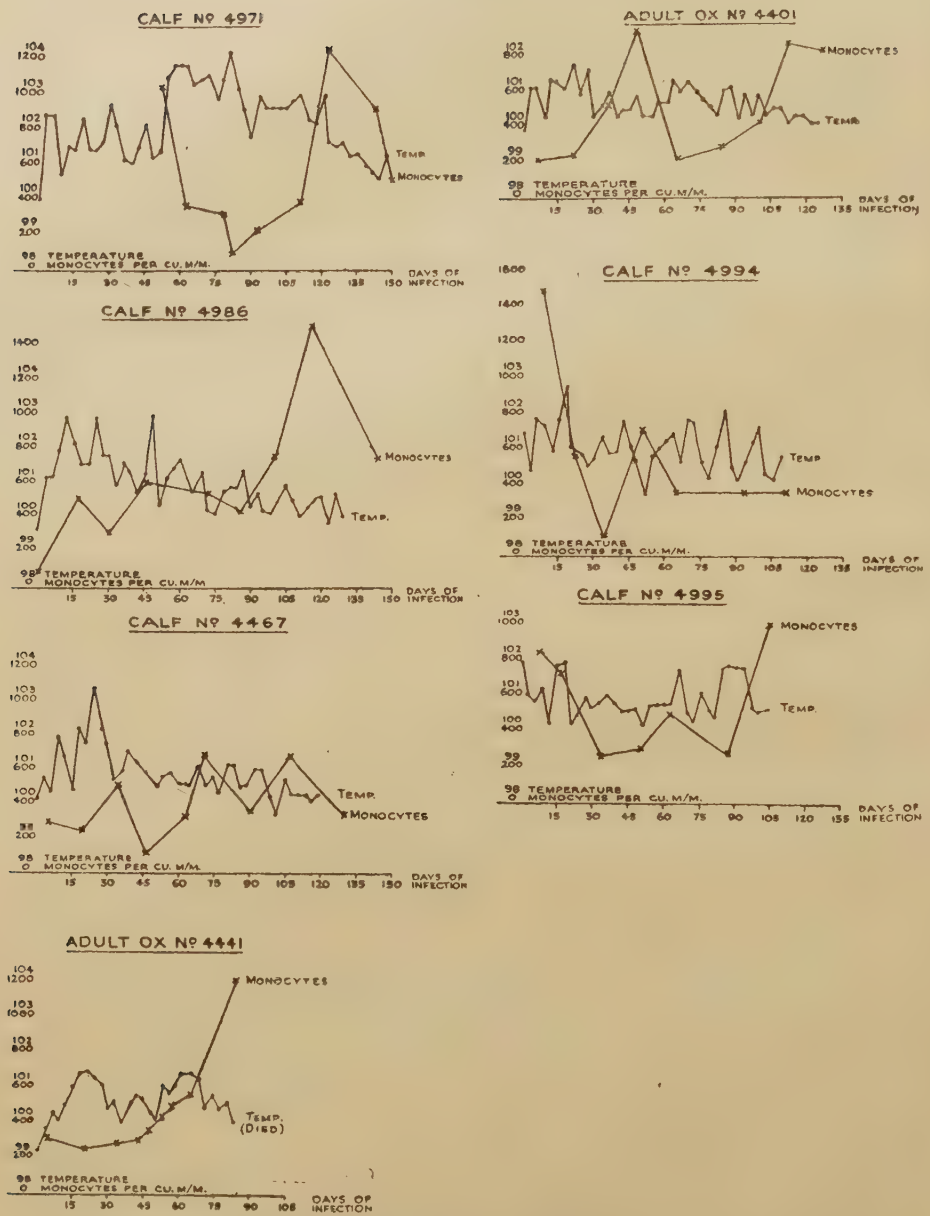
THE HABITAT OF THE TRYPANOSOMES

It was previously stated (*loc. cit.*) that the trypanosomes were never found in the tissues in nests or islets, as in the case of other pathogenic trypanosomes. The study of the blood vessels, however, showed that neither were they to be found in them. It was impossible that they could have been overlooked or had disintegrated with exceptional rapidity, because warm living tissue from animals destroyed *in extremis* had been used on most occasions and fixation was done in formol saline, Bouin, Zenker, Zenker without acetic acid and in Helly's solution; sections stained with Giemsa were also carefully searched, without one single trypanosome being discovered.

It was, therefore, essential to discover where the trypanosomes could be found, before the defence mechanisms were studied.

Trypanosomes are always more numerous in blood from the capillaries or superficial vein of the ear than from the jugular vein; they are also more numerous in the cool of the early morning than in the heat of the day. It seemed probable, therefore, that they lived chiefly in the superficial vessels of the skin and that the internal temperature of the body was too high for multiplication or survival. If the destruction of trypanosomes over a period was very great, this would not necessarily negative the theory that mobilisation of macrophage reserves was on such a scale as disastrously to deplete the endothelial reserves of the blood vessels; but it would be necessary to

Fig. 1.



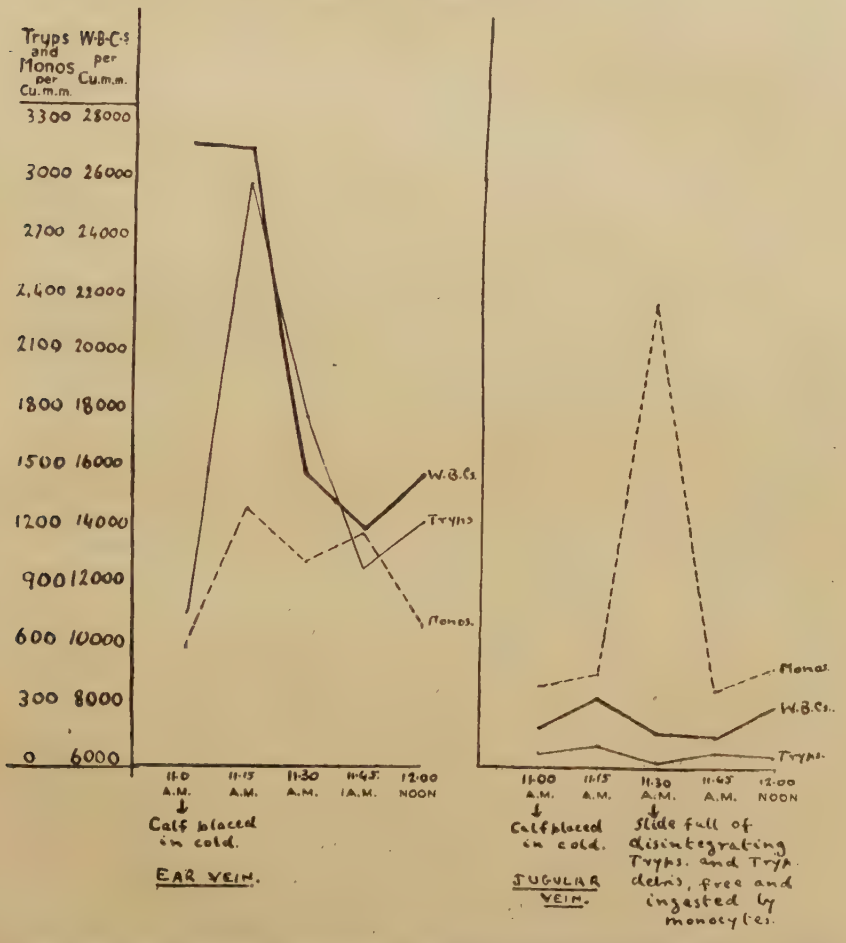
The temperature—monocyte relationship in cattle infected with *T. congolense*.

prove that destroyed trypanosome debris was in fact passed in considerable quantities into the systemic circulation.

Evidence of these matters could be found if the skin capillaries were constricted so as to drive the trypanosomes into the blood stream. The following experiment was, therefore, devised :—

Experiment 1.—A calf approaching the crisis stage of *T. congolense* infection and showing numerous trypanosomes in the peripheral blood, was placed in a cold room at a temperature of 4° C. for one hour; blood was drawn from the ear vein and the jugular vein at the beginning of the experiment and every quarter of an hour. Total and differential white cell and trypanosome counts were done on each blood sample. Results are shown in Fig. 2.

FIG. 2. CALF NO. 5455



White cell, monocyte and trypanosome counts under influence of atmospheric cooling.

In the jugular vein blood, neither trypanosome nor total white cell counts showed any significant variation, but in the ear blood, white cells initially at the high level of 27,000 per c.mm., dropped in half an hour to a more normal figure of 16,000 and showed no great variation thereafter. Trypanosome counts in the ear blood rose in quarter of an hour from 800 per c.mm. to 3,000, then dropped in the next two periods, successively to 1,800 and 1,000 per c.mm. Since trypanosome numbers remained constant in the jugular vein blood at a level of about 100 per c.mm., it is evident that a continuous rate of trypanosome destruction at a level of 1,000 to 3,000 per c.mm. of blood passing from the ear vein to the jugular vein has been maintained during the period in question. The debris from these destroyed trypanosomes has been ingested by cells of macrophage type, thus accounting for their migration into the blood stream in such large numbers.

In the ear blood, differential counts showed that, although the total white cells were reduced in numbers, there was some increase in the numbers of monocytes which corresponded to that of the trypanosomes; thus the peak occurred at the quarter-hour period. In the jugular vein blood, monocytes increased at the half-hour period from 2 per cent. to 40 per cent. of the total white cells, returning again to normal at the 45-minute period; this represented an increase from 450 of these cells per c.mm. of blood to 2,400, with a corresponding drop in the numbers of lymphocytes.

Examination of the half-hour slide from the jugular vein showed these cells to be in a state of tremendous activity. The slide was littered with granular debris, which could be shown as of trypanosome origin, since all stages between the intact trypanosome and the debris were traced. The monocytes were of all shapes and sizes, mostly full of granules and many had ingested polymorphs. The 45-minute jugular vein slide showed a monocyte with a half ingested intact trypanosome and the 15-minute ear blood slide, in which monocyte activity was much less, showed several more of these (Fig. 3), together with one monocyte fixed in the act of ingesting a polymorph, which had itself half ingested a trypanosome (Fig. 4).

The calf was again treated in the same manner, and jugular vein blood was taken at the half-hour period; this was examined stained by Giemsa and under supra-vital stain (neutral red). In the fresh preparation only two living trypanosomes were found in four whole slides, whereas they were numerous in the stained preparation. It was evident from this that the trypanosomes were either immobilised or dead, since in the fresh preparation it is very difficult to see trypanosomes that are not motile. Granular debris, confirmed from the stained preparation to be of trypanosomal origin, was present in considerable amount and was seen to be ingested by both polymorphs and monocytes, which were 47 per cent. of the white cells. The polymorphs were fairly active, but the monocytes had to be watched for some time to see the act of ingestion. After about three hours, most of the debris in the slide had been removed. The results of the experiment were somewhat disappointing, since the activity of the phagocytic cell was certainly not such as might have been expected from the striking appearances in the first part of the experiment.

Again the calf was treated in the same manner and a quarter-hour blood sample taken from the ear, since it was supposed that the jugular vein phagocytes might already have become inactive from attack on the trypanosome but would in the ear show greater avidity.

In the fresh preparations, trypanosomes were present in very large numbers and in a great state of activity ; there was little debris. Polymorphs were very active in attacking the trypanosomes, which got away from them on every occasion. Monocytes were present in fair numbers, at the beginning difficult to recognise as they contained very few granules and were not very active. Little was seen that showed the mechanism of phagocyte attack. One slide was then placed in the incubator at 38° C. for three minutes, but this appeared to revive the trypanosomes, which by that time were becoming more sluggish. A second slide was then placed on a hot plate at 40° C. for one minute. This caused all the trypanosomes to become either immobilised or very sluggish. They revived in about 20 minutes, but only survived some two hours against over four and a half hours in the control. At the end of the two-hour period monocytes were loaded with granules. When immobilised or relatively inactive, the trypanosomes were attacked with great persistence by the polymorphs. Usually the polymorph was unable to pick up the trypanosome and after a struggle released it ; thereafter, the polymorph appeared to die, while the trypanosome, which under attack showed some signs of life, died too or was ingested by another polymorph ; no monocyte was seen to ingest an intact trypanosome, though two were seen attacking a dead polymorph.

This experiment proved conclusively, firstly, that the main habitat of the trypanosomes is the superficial blood vessels of the skin, and secondly, that when they passed to the interior of the body they were quickly disposed of by the body defences at the normal blood or fever temperature of cattle.

Another important observation was made, which might alter present theories as to the habitat of the trypanosome in the body. In ear blood under supra-vital staining, amongst many hundreds of trypanosomes, one form was seen undoubtedly leishmanoid. The body was circular and its diameter slightly greater than that of the normal trypanosome ; there was no sign of undulating membrane, but there was a long thin flagellum issuing from one pole of the body. No blepharoplast was seen under supra-vital stain, but the nuclear area showed the typical faint pink stain, which all *T. congolense* show supra-vitally with neutral red. While the finding of such a form in the blood is probably entirely fortuitous and it may possibly be some aberrant appearance of the normal trypanosome, it may also indicate that developmental forms of *T. congolense* will eventually be found in the tissues either cutaneously or subcutaneously. This is, however, incidental to the present investigation.

THE BODY DEFENCES AGAINST *T. CONGOLENSE*

In the experiment just described it is plain that the trypanosomes have been driven by capillary occlusion from a favoured site to a part of the body less suitable for their survival. There is a sudden flush of trypanosomes in the venous blood, followed quickly by a flush of the cells concerned in defence. The appearances in the half-hour jugular blood indicate, not an activity of the monocyte that nobody has ever supposed it to possess, but a transitory passage of trypanosomes and monocytes to the general circulation.

It is thus proved that trypanosome debris does pass into the general circulation, and could pass to the parenchymatous organs of the body. It is proved that phagocytic cells, either polymorphs or monocytes, attack the living trypanosome and that they can both kill and ingest them. The enormous numbers of cells of macrophage type produced in a short time to deal with them has also been shown. Ingestion of the living or intact trypanosome does not, however, seem to be the first means of defence, since ingestion was seen to be mostly of disintegrated trypanosome debris. It has been shown that the normal or fever heat of cattle immobilises the trypanosomes, and it is probable that at this temperature they cannot resist the body defences. It is probable, however, that there is some serological defence of the body, which at these temperatures is able to attack the trypanosome. Though this is not really relevant to the present enquiry, two experiments may be described, for the sake of completeness, which demonstrate the part played by some form of antibody present in the blood serum.

Experiment 2.—Supra-vital slides (neutral red and janus green) were prepared; on one was placed one drop of separated trypanosomes from mouse blood plus one drop of white cells from ox blood (obtained by centrifugation) plus one drop of normal bovine serum; on the other was placed the same, but serum from an ox that had harboured *T. congolense*, the same strain as from the mouse blood, replaced the normal serum. On the control slide, the trypanosomes survived for about three and a half hours; on the experimental slide, for only ten minutes.

Experiment 3.—Two supra-vital slides (neutral red and janus green) were prepared and across each was stretched a piece of mesentery from mice infected with *T. congolense*; under the cover slip of one was run a drop of normal bovine serum and under the other a drop of serum from an ox that had been infected for many months with the same strain of trypanosome. Trypanosomes were present in the capillaries of the mesentery in very large numbers. They were dead or immobilised in the experimental slide within ten minutes but survived in the control for more than three hours.

These experiments indicate that the first line of defence against the trypanosome is serum antibody and temperature. The polymorphs and monocytes then attack the immobile, dead or disintegrated trypanosomes and ingest them; the polymorphs die quickly after attack and are ingested by the monocytes. The end point is thus the monocyte or macrophage, as had been deduced from the pathological findings.

DISCUSSION

The mode of attack in all trypanosomiases has been a matter of considerable controversy. The habit of most trypanosomes of congregating in the tissues has led to the supposition that the lesions are in general due to mechanical damage by the parasite, but this has never been accepted as the full explanation. In Chagas disease, for instance, the thyroid is seriously damaged but trypanosomes are

never found in the tissue. The toxic theory of action has been generally scouted in recent years, because of the failure of any worker to demonstrate a toxin.

T. congolense is here shown to be a parasite which in cattle lives solely in the skin capillaries and yet causes serious parenchymatous lesions in almost all internal organs. While it is not disproved that there is a toxin responsible for the condition, the study of the blood vessels, so extensively thrombosed, makes it seem probable that the lesion is a multiple infarction. Final assessment of this must be left to more experienced pathologists than the writer. The cause of the thrombosis can, however, be ascribed to two lesions, firstly, a hyperplasia of the " tunica adventitia " leading to loss of elasticity and constriction of the vessels, secondly, to a metaplasia of the endothelial cells which pass into the blood stream with the general macrophage mobilisation ; in this way favourable conditions for thrombosis are created and there is an arrest of some red blood cells which adhere to the elastic tunic and become centres of thrombus formation.

Proof that the macrophage is the end point of the body's attack on the trypanosome has led the enquiry somewhat far afield. The results, however, are so striking as to admit of no doubt of the tremendous drain on macrophage reserves, involved in combating the parasite. In assessing the significance of the experimental results, which have shown a transitory flush of the defence cells in response to an artificially created flush of trypanosomes in the general circulation, the relapsing nature of the parasite's attack must be remembered. The previous work under reference has shown that there are three relapse periods, possibly four, followed by a prolonged period of crisis, before the body defences obtain control of the trypanosome ; if flush periods occur of the order demonstrated, in addition to a continual drain on reserves, there is nothing inherently improbable in the macrophage reserves being so seriously depleted as is here suggested.

The study of the body's defences against the trypanosome is incidental to the general enquiry, designed to explain the pathology ; it will not be discussed here in detail, as it will form material for further investigation. For the present, it must be accepted that the one leishmanoid form of the trypanosome observed was an aberrant form of the normal trypanosome, but it suggests that a careful search of the cutaneous and subcutaneous tissues may reveal developmental forms of the parasite there.*

SUMMARY

Extensive thrombosis of the smaller blood vessels is shown to occur throughout the parenchymatous organs of the bovine body in

* These have now been demonstrated. R. N. T.-W.-F.

T. Congolense disease. The lesion is suggested to be a multiple infarction, affecting many essential organs.

The vascular lesion is shown to be due to reticulo-endothelial mobilisation, so extensive as to deplete the endothelial linings of the blood vessels. Hyperplasia of the " tunica adventitia " is a contributory cause, by diminishing the elasticity of the vessels and probably producing constriction of them.

It is shown that the first line of defence against the trypanosome is serum antibody and temperatures above 38° C. Both polymorphs and monocytes attack the parasites in the general circulation, but their main action is to scavenge trypanosome debris created by the trypanolytic action of the serum.

The main habitat of the trypanosome is in the skin capillaries and removal to the general circulation results in their rapid destruction. Debris then passes into the body and is subjected to attack by macrophages. Macrophage reserves are so severely depleted by relapsing attack, that the vascular endothelium is dangerously diminished, thus leading to thrombus forming conditions.

The finding of one leishmanoid form of the trypanosome in supravital preparation of fresh blood raises the possibility that developmental forms of the trypanosome may be found in the cutaneous or subcutaneous tissues.

ACKNOWLEDGMENTS

I take great pleasure in acknowledging my indebtedness to Dr. K. C. Smithburn, of the Yellow Fever Commission, Entebbe, for advice and help. I also record my appreciation of the work done by Mr. S. G. Laws, F.I.M.L.T., who has been untiring in the preparation of material and carrying out cellular and trypanosome counts.

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APPENDIX I

THE VASCULAR LESION IN SOME ORGANS

No. 4441. *Lymphatic Gland.*

Adventitious coat of most vessels hyperplastic. Metaplasia of endothelial cells, which appear to become swollen, columnar and to break away. Thrombosis of smaller vessels and of " vasa vasorum." One haemorrhagic area shows macrophages, removing tissue debris.

No. 5034. *Adrenal.*

Blood vessels empty. Hyperplasia of adventitious coats of arterioles and venules. Some necrosis of " tunica media." Endothelium deficient in

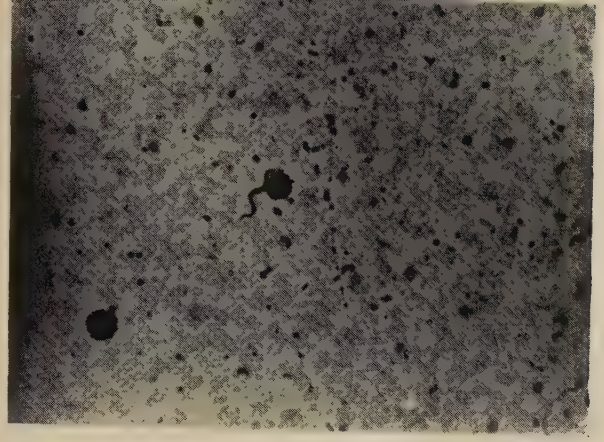


FIG. 3.

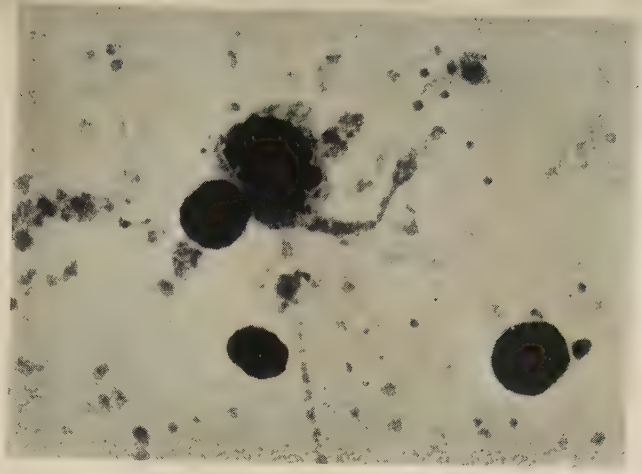
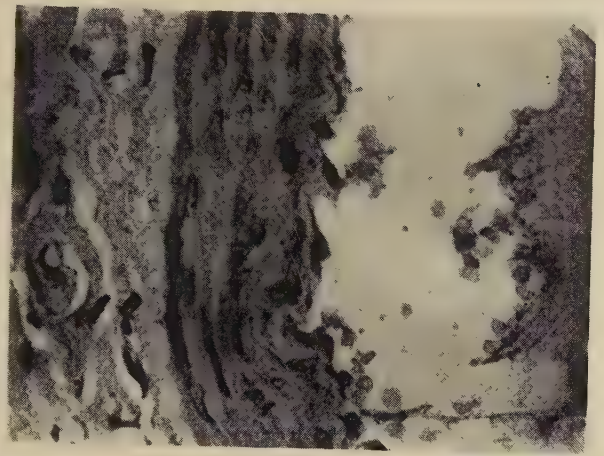
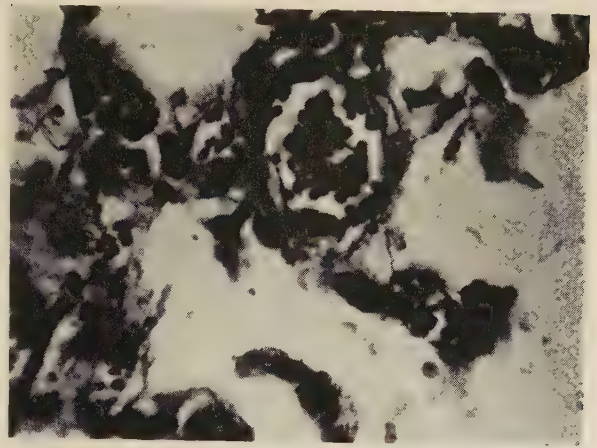


FIG. 4.





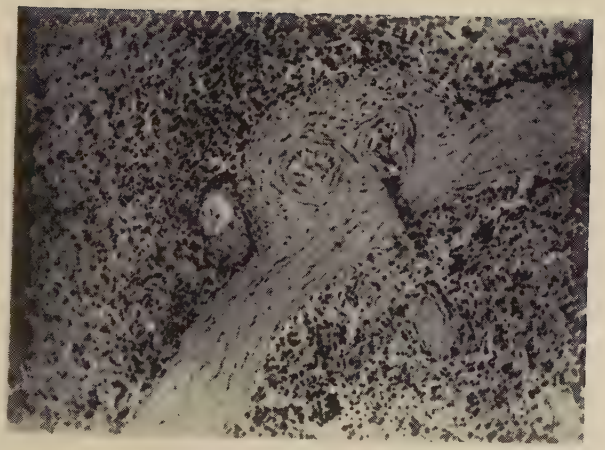


FIG. 7.

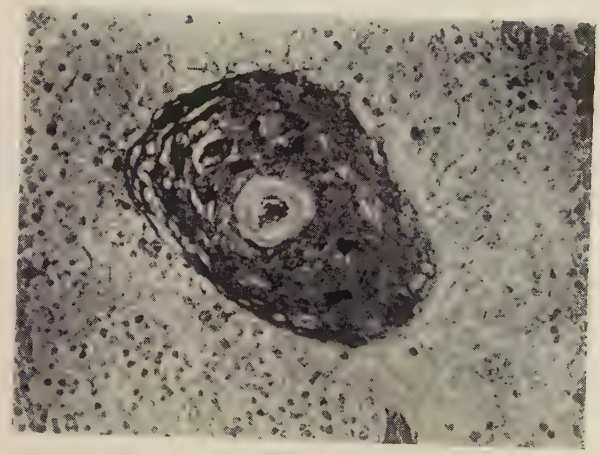


FIG. 8.

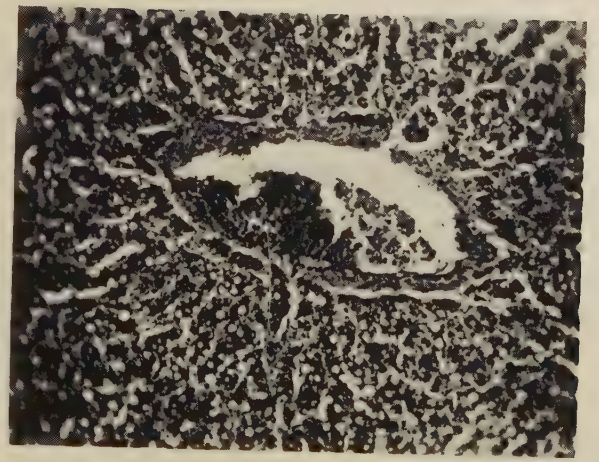


FIG. 9.

many places. Clasmatocyte type cells very numerous in the lumina of many vessels. Thrombosis of "vasa vasorum." The gland is necrotic and contains numerous haemorrhages.

No. 4971. *Spleen.*

Endothelium deficient from the smaller blood vessels. Arteriole walls appear necrotic.

No. 4874. *Liver.*

Multiple thrombosis of blood vessels throughout the organ, including one large branch of the portal vein. Branches of the hepatic artery show a swelling of endothelial cells, which are full of granules.

No. 4418. *Pituitary.*

The blood vessels of the capsule show hyperplasia of the adventitious coat. Clasmatocytes very numerous in them. "Tunica intima" shows metaplasia and hypertrophy of the endothelial cells.

N.B.—The above are samples, selected at random, of the appearances of the blood vessels, taken from different organs and from different experimental cases. They are given as being typical of the general appearances, seen almost without exception.

LEGENDS TO FIGURES

Fig. 3.—Trypanosome partially ingested by monocyte, x600. Thick smear from blood of jugular vein of calf, 45 minutes after placing in cold room at 4° C. Stained by Giemsa.

Fig. 4.—Trypanosome partially ingested by polymorph, the polymorph and trypanosome ingested by monocyte, x1200. Thick smear from blood of ear, 15 minutes after placing in cold room at 4° C. Stained by Giemsa.

Fig. 5.—No. 4954. Arteriole from lymphatic gland, x600. Shows swelling of some endothelial cells and below small developing thrombus of a few red cells. Haemalum and eosin.

Fig. 6.—No. 5034. Venule of lung, x600. Early stage of thrombus formation. Endothelial cells metaplastic and largely deficient. Red cells adherent to "tunica intima" and in one place the nucleus of a developing thrombus is present. Elastic tunic in one place stripped with effusion below. Haemalum and eosin.

Fig. 7.—No. 4954. Arterioles of spleen, x150. Thrombosis of three arterioles. Haemalum and eosin.

Fig. 8.—No. 1216. Arteriole of lymphatic gland x300. Thrombosis. Great hyperplasia of adventitious coat, in which are numerous large macrophages, some full of deeply staining granules. Giemsa.

Fig. 9.—No. 5043. Central vein of liver, x150. Fully organised thrombus. Severe fatty degeneration of parenchyma. Haemalum and eosin.

A STUDY OF THE PASSIVELY ACQUIRED ANTIBODY TO *TR. FOETUS* IN THE BLOOD OF YOUNG CALVES AND ITS BEHAVIOUR IN AGGLUTINATION TESTS AND INTRADERMAL REACTIONS

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INTRODUCTION

The immune reactions of cattle to the antigens of *Trichomonas foetus* during infection with the live organisms and in response to vaccination have formed the subject of previous communications (Kerr and Robertson, 1941, 1943).

In these investigations the blood response has been studied by following the development of agglutinins against *Tr. foetus*. It has been shown that the antigen is absorbed from the uterus and the antibody titre in the blood is a response to the absorbed antigen. It has also been observed that, under certain circumstances, during natural infections with living *Tr. foetus* and in cases where sterile Trichomonas antigen has been introduced into the uterus, this organ becomes sensitised so that further instillation of antigen at this site produces a systemic reaction considered to be of an anaphylactic nature. The reaction was held to be due to the fixation of antibody probably in the uterine lining and in other situations. Naturally infected animals and vaccinated animals have been found by one of us (W. R. K.) to develop a skin reaction to the intradermal injection of an extract of *Tr. foetus*. This has been found of practical value in diagnosis and has been a useful tool in the further elucidation of the antibody reaction. It is still being investigated (Kerr, 1944).

In addition, a preliminary account has been given of the direct absorption of Trichomonas antibody by newly born calves from the colostrum of cows with high titre sera. The transference of antibody in the colostrum via the alimentary canal is of interest for many reasons ; in particular, it affords an excellent example of a circulating antibody acquired passively by a natural physiological process.

It is obvious that all these different reactions to *Tr. foetus* antigen are very intimately connected, but for the sake of clarity in exposition and convenience they are divided up under separate headings. The present paper is an account of investigations carried out with calves and deals mainly with observations on passively acquired Trichomonas antibody in young bovines.

METHODS AND MATERIALS

The blood tests are carried out by the direct agglutination of a living suspension of *Tr. foetus* organisms. The method has been described in detail in previous communications (Robertson, 1941; Kerr and Robertson, 1941, 1943). One important feature must, however, be recalled as it has a bearing on the work, particularly in young animals. The serum of adult normal bovines agglutinates living *Tr. foetus*. This normal titre is, in the opinion of the writers, due to the circulating serum proteins (probably globulins) in adult bovine serum which combine to a certain extent with living Trichomonas; it is not specific in the sense that it is produced by the Trichomonas antigen. The sera of acutely infected or vaccinated animals show agglutination of greater intensity and to a higher dilution than those of normal animals.

The titre of the agglutinins in colostrum is measured by using centrifuged and filtered whey derived by treating the colostrum with rennet. Though the normal agglutinin will be considered to some extent in this paper it is still the subject of further study.

The extracts used in the skin tests have been saline extracts, trichloracetic precipitated extracts and laked bodies of well-washed Trichomonads. Whether these extracts are absolutely specific when used in animals harbouring other infections and in those sensitive to various proteins is still being investigated. The value of the skin test in carefully controlled experimental work in animals whose whole history is known is very great.

It should be appreciated that there are very considerable practical difficulties in experiments with newly born calves.

For this reason in the experiments about to be described the calf was always removed from the cow at the moment of delivery and a blood sample was immediately taken from the calf in order to ensure that it had not contrived to have an illicit feed while other matters are being attended to.

EXPERIMENTAL RESULTS

Colostrum fed calves of normal mothers

The sera of newly born calves of normal mothers contain no agglutinins for *Tr. foetus* in a dilution of 1/6 always provided the sample is taken at birth before the young animal has sucked its mother. After the calf has been fed on the colostrum the serum may acquire an agglutination titre which, though it varies very considerably, is nearly always below the titre of the normal adult blood.*

* When this paper was already in the press we came across Pedersen's pamphlet (Pedersen, 1945) in which by means of the ultracentrifuge he discovered a new low-molecular globulin which he called "fetuin." This is present in the foetal serum of calves to the almost complete exclusion of the ordinary globulins and antibodies which only appear after the animal has received the colostrum. The bearing of this on the inert character of the serum of newly born fasting calves to *T. foetus* is obvious and is an extension and confirmation of Howe's much earlier findings. (Howe, 1921.)

The agglutinin may be very evanescent or it may persist for the first few weeks, but the titre has nearly always dropped almost to nothing by the end of the third or fourth week of life. The amount of agglutinin found in the sera of these young colostrum fed animals seems to depend upon the titre of the whey of the colostrum.

As the young calf develops a natural agglutinin for *Tr. foetus* is gradually produced. In most calves there is a period from about the fourth to the eighth month during which the normal titre is developing and the sera give curiously uneven agglutination results. The reason for this is not clear ; it has, however, been observed over and over again. The titre not only varies but also does not seem to depend consistently on the concentration of the serum. Samples are also found in which incomplete agglutination occurs, that is, clumping takes place but is never complete. In calves of normal mothers the development of the normal agglutination titre has never, in the experience of the writers, been accompanied by the appearance of skin sensitiveness to the intradermal injection of Trichomonas extract.

Colostrum fed calves of high titre mothers

In these observations the calves have been produced by mothers vaccinated with Trichomonas antigen previous to being put in calf or during pregnancy. At birth the serum of the calf is without agglutinins for *Tr. foetus* just as in the calf from a normal mother, but after ingesting the colostrum it acquires an agglutination titre approximating that of the whey of the colostrum. The titre of the colostrum is usually as high as—in some cases higher than—that of the cow's serum. (A9, Table I.) The absorption of the antibody from the colostrum is very rapid. In one example, two hours after the first feed, the serum of the calf (B15 calf) had a titre practically equal to that of the whey.

Though the agglutination test is not an accurate quantitative measure, it gives a fairly good idea of the relative antibody content. So rapid is the absorption that it would seem that the antibody-bearing proteins and also the globulins responsible for the non-specific agglutinins must pass with very little alteration directly into the circulation of the calf. In some cases the absorption is a little slower, but in 12 to 16 hours the peak titre of the serum of the calf is usually reached. The antibody-bearing proteins in the whey of the colostrum are obviously not representative of the total serum proteins of the maternal serum and, as will be shown later, the elements in the whey seem to be more readily absorbed.

The passive antibody titre of the calf's serum gradually falls but the rapidity of the loss varies very considerably. It does, however, drop in all cases and there is usually, though not quite always, a period when the serum has a low titre, well below the normal of the adult animal.

In these passively immunised calves a period of irregular agglutination may also appear, during which the tests are difficult to read and are of doubtful value. The serum of calves, moreover, even after they have ceased to be fed on milk, is often very full of lipoid material which seems sometimes to interfere with the agglutination test.

The elimination of the passive immune body acquired via the alimentary canal does not run parallel to the usually accepted rapid elimination of foreign serum from the blood stream. The conditions are clearly not entirely similar. In the calf it is difficult to establish the exact period at which one estimates that the passively acquired antibody has disappeared, as, up to the present, it is not possible to distinguish in the agglutination tests the passive immune titre from the agglutinin which normally develops in all these animals. Nevertheless, the passive titre has, in many cases, sunk to a level which is below that of the normal adult, and is at about the expected titre of the developing normal titre by the fourth month. It should be understood that this level can, of course, be produced in a young calf if a low titre colostrum is ingested or indeed if a low or medium titre whey is given as the first feed. (Table I, B16.)

The skin reaction gives valuable information in regard to the nature of the circulating agglutinin. After a time which varies considerably but which, in one instance, was as short as ten days (A11 calf, 1943) after the colostrum feed, calves which have ingested high titre colostrum and have acquired a passive antibody circulating in the serum, were found to have a positive skin reaction to intradermal injection of the antigen. This indicates that the circulating passive antibody can become quickly fixed in the skin.

There is a definite quantitative connection between the titre of the calf's serum and the titre of the whey of the colostrum and a somewhat less obvious connection between these and the duration of the titre and the appearance and duration of the skin sensitiveness. The latter is brought about in a purely passive way and thus it may be regarded as a fixation of antibody which is, or has been, circulating passively in the blood stream. An example of the quantitative relation is afforded by calf B16 (Table I). The mother was vaccinated with minimal amounts of Trichomonas extract and the titre of the maternal serum at birth was about the normal (11 : 9·5). The titre of the whey of the colostrum was also 11 : 9·5. The serum of the calf at birth had no agglutinin, but at 12 hours after the colostral feed it had a blood titre of 8·5 : 9·5—that is, it was at the normal adult level. The serum of the calf, however, contained Trichomonas antibody and while the blood titre dropped to 4·5 in three to four weeks, the skin test was positive up to three and a half months although it was negative at seven months.

All our observations have so far shown that the normal agglutinin does not cause a sensitive state in the skin whereas the Trichomonas specific agglutinin does. The matter of the distinction of

the two agglutinins requires further study but this work on the calves makes it clear that there are two different types of reaction to the presence of the agglutinins. In calf B16 (Table I) the agglutinin is never above the normal adult level, but it differs from the normal agglutinin in that it becomes fixed in the skin and reacts to the Trichomonas antigen. The skin remains sensitive as a rule for a much longer period than that during which a Trichomonas blood titre can be detected by the agglutination method. Its duration, too, varies according to the amount of antibody originally present; in animals with a low intake it may have disappeared in four to eight months (B16, Table I), or it may, in other cases, persist up to 20 months (A11 calf, 1942).

Ingestion of colostrum containing Trichomonas antibody by the calves of normal mothers

In the cases just described the calves were the offspring of vaccinated mothers and it might be objected that their reactions would, on that account, be different from those of calves derived from normal mothers. Although calves of vaccinated mothers have never so far shown agglutinins in the blood at birth, it was thought important to meet this criticism.

As it is very difficult to have the fresh colostrum of a vaccinated cow available at the moment of birth of a normal calf, whey preserved in the ice chest from the colostrum of a vaccinated mother was fed to the new-born normal animal. The calf of a normal cow was removed at birth from its mother. After taking the first blood sample it was given as its first feed, when it was six hours old, $1\frac{1}{2}$ pints of boiled milk mixed with $1\frac{1}{2}$ pints of whey containing Trichomonas antibody (titre 15 : 9) from the colostrum of a vaccinated cow. (B17, Table I.) The serum of the calf when tested for agglutinins two hours after the feed, had a titre of 2·5 : 9·5 showing it had absorbed some of the immune body in that short time. In 24 hours the titre was 9·5 : 9 and at three days it was 11 : 9. The agglutination titre dropped again to 3·5 : 9 in three months. The skin was sensitive to Trichomonas antigen intradermally on the 39th day (it was not tested earlier) and remained positive for four months, but after an interval during which the reaction was doubtful it became negative at eight months.

The normal calf, therefore, was able to absorb the antibody and behaved in all respects exactly like B16 calf, 1943 (Table I) where a low antibody content was passed on to a calf from a vaccinated mother by means of the colostrum.

Conditions of absorption of the antibody from the whey

The conditions under which the newly-born calf can absorb the serum globulin carrying the antibody from the colostrum have been examined. Two obvious questions present themselves. Can

the newly-born calf absorb the antibody only with the first feed? For how long can it absorb the antibody? The separation of time alone as a factor was not investigated owing to the general bad effect of withholding food from the very young animal. It had been previously ascertained that the antibody appears to be completely destroyed by boiling and that calves receiving boiled milk show no agglutinins in the serum during the first weeks of life. (Table I, calf B42.)

It was found (A3 calf, 1943, Table I) that a calf given boiled milk when four hours old and then fed on the maternal colostrum with a whey titre of 15·5 : 9·5 when 12 hours old, showed a titre of 8·5 : 9·5 in its serum at 24 hours, falling rapidly, however, to 7 : 9 four days later and 3·5 : 9 in 20 days. This calf had shown a partial absorption of the antibody at 12 hours when it had had one previous feed of boiled milk. The skin test was positive at ten weeks (not tested earlier). But when a newly-born animal (B19 calf, Table I) was fed for 24 hours on boiled milk and then given colostrum with a whey titre of 18 : 9·5 it failed to absorb any appreciable amount of antibody and the serum showed no agglutinin for *Tr. foetus*. This calf on repeated testing never developed a skin sensitive reaction. These results are in agreement with those obtained by Mason, Dalling and Gordon (1930) in their study of the absorption of antibody in lambs.

Transference of antibody direct from serum

In the foregoing sections the passive absorption of antibody from the colostrum via the alimentary canal has been described. There arises the closely allied question as to whether the calf can absorb the antibody directly from the serum through the alimentary canal. This is a purely academic point but is of theoretical interest. It was found that calves do not tolerate undiluted serum as a first feed very well but the following results were, nevertheless, obtained on two calves.

The first (calf B35, Table II) was the offspring of a normal mother and at the age of about an hour it was fed with 20 oz. of high titre serum. Four hours later a blood sample was taken and boiled milk was given until milk feeding was discontinued. At birth the serum was, as usual, without agglutinin, but four hours after the feed on the high titre serum the agglutinin titre was 6·5 : 9, at 14 hours it was 12 : 10, and in three days it was 13 : 9. The titre of the serum ingested was 20 : 9·5 and the calf serum, though showing the absorption very clearly, never reached that titre, suggesting that the antibody is in a less easily assimilated form in undiluted serum than when present in the whey (A9, Table I). The duration of the titre was similar to that in experiments producing a similar response when whey was used. It began to drop after a fortnight and had declined to 4·5 : 9 in six weeks. The fixing of the antibody in the skin did not occur, as was the case in the whey-fed calves, since the skin

reaction remained negative up to 90 days. When tested at five months old, however, the skin reaction was positive; it was negative again at eight months.

In the control animal (B37, Table II) the ingestion of normal serum under similar conditions produced only the slightest trace of agglutinin, the titre being at its highest, 1 : 9·5.

This aspect of the work was not pursued any further.

The injection of serum containing antibody subcutaneously into young animals

Twenty ounces of high titre serum (19 : 9) was injected subcutaneously into a ten-day-old calf, the offspring of a normal mother (B38 calf, 1944, Table II). The serum contained no agglutinin for *Tr. foetus* up to the time of the injection of the serum, but within 24 hours the titre rose to 15 : 9. The skin test gave a positive reaction eleven days after the serum was injected.

A normal newly-born calf (B40, Table II) fed on boiled milk for two days and then on fresh milk had no agglutinins for Trichomonas in the serum on the ninth day. Twenty ounces of a normal serum with a non-specific titre of 8·5 : 9 were then injected subcutaneously. After a further 48 hours the titre of the calf's serum was 2·5 : 9. The titre dropped gradually and was only represented by a trace in 23 days, but in six weeks rose to 5 : 9, suggesting the beginning of the development of the normal non-specific agglutinin. The skin test was negative eight weeks after the injection of the normal serum.

DISCUSSION

The experiments recorded above are of interest chiefly on account of the light they throw on the following points: the absorption of antibody from the alimentary canal; the fixation of passive antibody in the skin; the distinction between normal and specific agglutinins; and the theoretical similarity between the action of antibody in high titre serum and in the colostral whey.

The very rapid absorption of the antibody from the colostrum is of importance in showing that, although it would seem that the calf does not receive antibody *in utero*, it makes up for this by acquiring the antibody circulating in the maternal serum from the colostrum by way of its alimentary canal. The results mentioned here are in close agreement with those of Mason, Dalling and Gordon (1930) in lambs and show that the conditions in the alimentary canal which permit of the direct passage of the antibody disappear within the first 24 hours. The state of the serum of the calf in regard to the globulin content is of the greatest interest and requires further investigation. Howe (1921) showed that the serum is very poor in globulins at birth and that these substances are acquired from the colostrum, but there is very little information about the fate of the

ingested serum proteins or the subsequent development of these substances as life progresses.

The development of the normal non-specific agglutinin is not complete till somewhere between the fourth and eighth months. Its appearance is irregular and there is some evidence from the variable results obtained in agglutination tests during this period suggesting that it is at first not consistently effective in its action on living *Tr. foetus*. The nature of the normal agglutinin is not clear, but it would appear to come under the heading of a normal character or property of the bovine serum. In this connection Hirszfeld's (1926) study of the blood agglutinins in human sera is interesting as giving examples of highly active substances (antibodies ?) which react upon antigens which were not concerned with their production.

The normal agglutinin for *Tr. foetus* is a natural character of the serum of all normal adult bovines ; it combines with living Trichomonads but it is not produced by Trichomonas antigen. It is not fixed in the skin and does not react with the antigen of Trichomonas injected intradermally. The specific Trichomonas agglutinin combines with living *Tr. foetus* and is produced in response to the presence of Trichomonas antigen. The antibody becomes fixed in the skin and reacts with the Trichomonas antigen after intradermal injection to produce a positive skin test. A considerable amount of information is given as regards the nature of the skin reaction by these feeding experiments in young calves. The circulating antibody which is produced in the mother and is taken over quite passively by the calf is not connected with infection by the organism or with any active response in the calf to the Trichomonas antigen. The evidence is therefore clear that the skin is capable of being used as the repository of the antibody and that the result of this dermal fixation is the power to react to the subsequent injection of Trichomonas antigen.

The word allergy has been so widely and variously applied to a number of sensitive states that its meaning is obscure ; moreover, skin sensitiveness in general may be brought about by several different mechanisms (Harington, 1944). The present writers do not wish to use it for the case under discussion. They suggest that the sensitiveness revealed in the skin test is quite clearly due to fixed antibody and might be considered to be a minor anaphylactic reaction.

It is interesting to note in conclusion that the Trichomonas antibody in serum can be absorbed via the alimentary canal into the blood stream in the newly-born calf though it is much less easily assimilated in this vehicle than in the whey of the colostrum. The fixation of the antibody in the skin is delayed and the skin test does not become positive till between the third and fifth month. The significance of this delay cannot be estimated, however, from a single experiment.

Following the subcutaneous injection of high titre serum the antibody can also pass into the blood stream of the calf and in this

case may apparently become fixed in the skin. It is, however, quite clear that the ingestion of the colostral whey at birth is (apart from being the natural path) the most efficient method of transferring the maternal antibody into the serum of the calf.

It has been assumed in this work that the antibody to Trichomonas antigen is situated in the globulin, but there is at present no direct knowledge that this is so. It would be of interest to explore the serum fraction in which the various antibodies are situated in bovine sera.

SUMMARY

The foregoing paper deals with experiments in new-born calves showing the absorption of the antibody against *Tr. foetus* from the maternal colostrum into the blood of the calf by way of the alimentary canal in the first hours of life; the fixation of the passive antibody in the skin; the distinction between normal and specific agglutinins; and the theoretical similarity between the action of antibody in high titre serum and in colostral whey.

The antibodies against *Tr. foetus* present in the whey of the colostrum can be absorbed by the new-born calf at the first feed after birth, and to some extent at 12 hours after birth, the calf having received boiled milk as its first feed at four hours old. If the calf has been fed on boiled milk for 24 hours the antibody present in the colostrum is not absorbed.

The antibody passively acquired by calves reacts in agglutination tests with the living Trichomonas. It also becomes fixed in the skin and reacts with Trichomonas extract producing a positive skin reaction.

The normal non-specific agglutinin for *Tr. foetus* is present in the serum of all adult bovines; it combines with living Trichomonas but it is not produced by Trichomonas antigen. It is not found in the blood of the new-born calf, but arises during the first six months of life. The non-specific agglutinin does not become fixed in the skin and does not react with the antigen of Trichomonas extract in intradermal injection.

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TABLE I

BLOOD TITRE AND SKIN REACTION (WHERE AVAILABLE) OF CALVES

Titre of Cow at Time of Calving		*Titre of Calf at Birth Before First Feed*	*Titre of Calf After Feeding*		*Titre of Calf During Period of Growth*							
Titre of Blood	*Titre of Whey*		*On Colostrum of Mother*	*On Boiled Milk*	*2–7 Days*	*2–3 Weeks*	*4–6 Weeks*	*2–4 Months*	*5–7 Months*	*8–12 Months*	*12–18 Months*	*After 18 Months*
A7 1942 normal $9\frac{1}{2} : 9\frac{1}{2}$	—	—	Trace at 24 hours	—	—	Skin test constantly negative						
B44 1945 normal 9 : 9	$7\frac{1}{2}$	0	$2\frac{1}{2} : 9$	—	2 : 9	$0 : 9\frac{1}{2}$	$2\frac{1}{2} : 9\frac{1}{2}$	4 : $10\frac{1}{2}$	$8\frac{1}{2} : 9\frac{1}{2}$	$8\frac{1}{2} : 9\frac{1}{2}$	$9\frac{1}{2} : 9\frac{1}{2}$	$9\frac{1}{2} : 9\frac{1}{2}$
B42 1945 normal	—	0	No colostrum	Boiled milk	0 : 9	—	—	—	—	—	—	—
A9 1943 $19\frac{1}{2} : 9$ vaccinated	20 : 9	0 : 9	$18\frac{1}{2} : 9$ at 13 hours old	—	16 : 10	14 : 10	8 : $9\frac{1}{2}$	9 : $9\frac{1}{2}$	—	$12\frac{1}{2} : 10$	$9\frac{1}{2} : 9$	—
B16 1943 $11 : 9\frac{1}{2}$ vaccinated minimal doses	$11\frac{1}{2} : 9$	0 : 9	$8\frac{1}{2} : 9\frac{1}{2}$ at 12 hours old	—	$8\frac{1}{2} : 9\frac{1}{2}$	Skin test positive up to 13 months $4\frac{1}{2} : 9$	—	6 : 9	—	$9\frac{1}{2} : 9\frac{1}{2}$	—	—
B17 1943 normal cow	Titre of whey given with boiled milk 15 : 9. The whey is from another animal	0 : 9	No colostrum, boiled milk plus whey given at 6 hours old. Two hours after feed titre $2\frac{1}{2} : 9$	—	$9\frac{1}{2} : 9$ first day 11 : 9 third day	Skin test positive at $3\frac{1}{2}$ months, negative at 7 months —	—	$3\frac{1}{2} : 9$ 5 : $9\frac{1}{2}$	— 9 : $9\frac{1}{2}$	— $9\frac{1}{2} : 9$	—	—
A3 1943 $12\frac{1}{2} : 9\frac{1}{2}$ vaccinated	$15\frac{1}{2} : 9\frac{1}{2}$	$0 : 9\frac{1}{2}$	Fed at 12 hours old 2 pts. of mother's colostrum. $8\frac{1}{2} : 9\frac{1}{2}$ at 24 hours	At 4 hours old given boiled milk as first feed	Skin test positive up to 4 months, then doubtful; negative at 8 months 7 : 9 4 days old	$3\frac{1}{2} : 9$	3 : 9	2 : 9 4 : 9	—	—	—	—
B19 1943 vaccinated $15\frac{1}{2} : 9$	$18\frac{1}{2} : 9$	$0 : 9\frac{1}{2}$	Given colostrum at 24 hours. 1 hour later titre $0 : 9\frac{1}{2}$	Fed for 24 hours 0 : $9\frac{1}{2}$ at 18 hours	$0 : 9\frac{1}{2}$	$1\frac{1}{2} : 9\frac{1}{2}$	Skin test positive at 10 weeks —	—	—	—	—	—
							Skin test remained negative					

TABLE II

BLOOD TITRE AND SKIN REACTION (WHERE AVAILABLE) OF CALVES FED OR INOCULATED WITH SERUM

History of Calf	*Titre of Serum Used in Experiment*	*Titre of Calf at Birth*	*Titre of Calf After*		*Titre of Calf During Period of Growth*					
			Feeding on Serum	*Injection of Serum*	1–7 *Days*	2–3 *Weeks*	4–6 *Weeks*	2–4 *Months*	5–7 *Months*	8–12 *Months*
B35 newly born calf of normal mother	20 : 9 high titre serum from vaccinated animals	0 : 9	20 oz. of high titre serum given at first feed. $6\frac{1}{2}$: 9 in 4 hours	—	12 : 9 13 : 9	10 : 9	$7\frac{1}{2}$: 9	$4\frac{1}{2}$: 9– $3\frac{1}{2}$: 9	—	—
					Skin test still negative at 3 months ; positive at 5 months ; negative at 8 months					
B37 newly born calf of normal mother	9 : $9\frac{1}{2}$ normal serum	0 : 9	20 oz. of normal serum given as first feed. 1 : 9 after 4 hours	—	$1\frac{1}{2}$: $9\frac{1}{2}$ 24 hours $\frac{1}{2}$: $9\frac{1}{2}$ 3 days	$\frac{1}{2}$: 9	1 : 9	—	—	—
					Skin test negative at 3 weeks					
B38 newly born calf of normal mother fed on boiled milk for 2 days then on unboiled milk	19 : 9 high titre serum from 2 vaccinated animals pooled	0 : 9 at birth 0 : 9 at 5 days 0 : 9 at 10 days	—	20 oz. high titre serum injected subcutaneously at 10 days old, 15 : 9 after 24 hours	$16\frac{1}{2}$: 9 3 days	$15\frac{1}{2}$: 9 after 9 days		—	8 : 9	—
					Skin test positive after 11 days					
B40 newly born calf of normal mother fed on boiled milk for 2 days then on unboiled milk	$8\frac{1}{2}$: 9	0 : 9 at birth 0 : 9 at 10 days	—	20 oz. normal serum injected subcutaneously at 10 days old	$2\frac{1}{2}$	1 : 9– $\frac{1}{2}$: 9	3 : 9	5 : 9	—	—
					Skin test negative at 10 weeks					

Footnote to Tables.—The " index " is the number of + signs given to express the degree of agglutination in the first four plates of the dilution series of the test serum over the number given for the normal control serum of the day. Owing to economy in printing only single typical protocols are given in the tables. References in the text are made to individual experiments not illustrated in the tables. Where the tables are referred to the Number of the table is given.

A RADIOGRAPHIC STUDY OF THE NORMAL DEVELOPMENT OF THE HOCK JOINT OF THE FOWL

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INTRODUCTION

In the course of routine assays of the antirachitic activity of various oils for young chicks in which the radiographic technique described by Olsson (1936) was used, the sporadic occurrence of certain unusual appearances of the metatarsal bones was observed.* It was at first thought possible that these appearances might be the precocious start of the changes in the hock joint which normally precede the union of the tarsal ossification centres with the tibia and metatarsus. Precise information about the nature and time of development of these changes was not found in the literature available at the time. It was therefore decided to examine the radiographic appearance of the hock joint of growing chicks by periodic X-ray photographs. Subsequently, two monographs (Olsson, 1941, and Nielsen, 1942), in which this aspect is described and illustrated, were procured.

Textbooks dealing with the anatomy of the fowl give few details of the hock joint of the growing chick. Bradley (1938), for example, states merely that in the embryo there are indications of two rows of tarsal elements, but the proximal row soon fuses with the tibia and the distal row with the metatarsus: he states that the proximal row contains two elements, the distal row one. Kaupp (1918) and Chamberlain (1943) give a similar description and also refer to the hypotarsus and hypotarsal canal and the hypotarsal sesamoid.

Hall and King (1931) state that the upper row of tarsals has already fused with the tibia in 12- to 14-weeks-old chicks, whereas fusion of the lower tarsal with the metatarsus has not yet taken place. Titus (1932) asserts that fusion of the tibia and tarsus begins at 12 weeks. Olsson (1941) gives a series of seven radiographs showing the development of the tarsal joint between one day after hatching and 24 weeks. He states (p. 50) that fusion of the tibia with the tarsus begins at four weeks, but earlier (p. 45) he refers to fusion beginning at one week. The radiographs purporting to show fusion at four weeks seem to show merely overlapping of the tibia and tarsals and not actual fusion: lateral X-rays are necessary to confirm the time of fusion. Nielsen (1942), who was apparently unaware of Olsson's work, believes that fusion of the tibia with the upper tarsal centres occurs

* The unusual appearances, observed at routine X-ray when the birds were four to five weeks old, were subsequently identified as the early stages of perosis (Bruce *et al.*, 1946).

at about nine weeks but that the epiphyseal line remains open until all growth in length of the bone has ceased and that the distal tarsal centre unites with the metatarsus at about 12 weeks.

METHODS

In the present study we have followed the development of the normal hock by means of weekly radiographs. The birds were housed under brooder conditions until they outgrew the brooders, when they were transferred to cages with wire mesh floors. Only restricted exercise was possible but the birds remained in excellent condition throughout the period of observation and growth was good, the birds weighing 2·5 to 3 kg. at 20 weeks. The ten birds on which observations were made were cockerels of Rhode Island Red–Light Sussex cross. For the first 8 to 12 weeks they received a basal diet similar to diet No. 1 described in the B.S.I. pamphlet No. 911 (British Standards Institution, 1940) with added vitamin D_3, after which they were given a grower's mash.

RESULTS

From the radiographs obtained, a series (Figs. 1 to 12) has been chosen to illustrate the sequence of events. Emphasis has been laid on those stages which illustrate the changes most clearly and no attempt has been made to standardise the intervals between successive stages or to select successive stages from pictures from one particular bird. The three radiographs of the normal adult bird (Figs. 10 to 12) were taken at the South-Eastern Agricultural College, Wye.

It will be realised that radiographs show the development only of the bony structures of the hock and do not reveal the condition of the cartilage matrix in which the centres of ossification are situated. In the newly hatched chick the tibiotarsus and tarso-metatarsus are similar in form to the corresponding bones of the adult fowl and the hock is a simple hinge joint. Ossification of the hock takes place from two centres in the distal end of the tibiotarsus and one in the tarso-metatarsus and these centres are considered to correspond to the tarsal bones of mammals but do not exist as separate tarsal bones. One ossification centre occupies each tibial condyle and the third forms the head of the tarso-metatarsus.

During the first eight weeks after hatching no change in the shape or proportions of the bones forming the hock was observed. Thus in a five-weeks-old bird (Fig. 1) the two proximal tarsal centres are distinct from the tibia but lie so close to it that a slight rim around the margin of the distal end overlaps them. The single distal tarsal centre is lens-shaped and separated from the metatarsals by a zone of uncalcified cartilage matrix. The metatarsus is composed of three bones, free from one another at the proximal end but united in the region of the shaft. The smallest bone lies

somewhat posterior to the other two and each bone has a well-defined and densely calcified proximal edge.

After eight weeks the medial upper tarsal increases in relative size and approaches the less rapidly growing lateral tarsal (Fig. 2). Within the next two weeks the gap between them disappears (Figs. 3 and 4) and the fused tarsal centres begin to unite with the tibia. Fusion proceeds from the centre outwards until at 12 to 14 weeks the tibiotarsus appears as a single bone (Fig. 7). At first a line of dense calcification marks the site of union but this becomes less conspicuous and disappears entirely at about 20 weeks (Fig. 9), leaving no sign of the triple origin of the tibiotarsus.

The formation of the tarso-metatarsus takes place less rapidly. At ten weeks, when the proximal tarsal centres are already fusing with the tibia, the metatarsus retains its distinct head and shows no sign of fusion with the lower tarsal centre, which extends only half-way across the joint (Fig. 4). As the centre increases in diameter and thickness, the gap between it and the metatarsus is lessened and the edge of the metatarsus becomes hazy (Fig. 7). With increased thickening, the tarsal becomes adapted on its proximal surface to articulate with the tibiotarsus and joins distally with the metatarsus (Fig. 8). Fusion appears to proceed from the centre outwards and is complete at about 20 weeks (Fig. 9). The densely calcified line of demarcation is still visible at one year (Fig. 10). Complete continuity of structure is attained in a two-year-old bird (Fig. 11). Fusion of the three metatarsals has not begun at about 14 weeks (Fig. 7), but is complete by one year (Fig. 10).

The lateral radiograph (Fig. 12) shows the features of the simple hinge joint of the tarsus in the adult. The hypotarsal sesamoid bone which develops over the medial ridge of the trochlea and gives rise to the shadows seen in this situation in Figs. 8 and 9 is seen posterior to the lower tibial epiphysis. The hypotarsus, a bony prominence, which contains a longitudinal groove and canal giving passage to the flexor tendons of the foot, is seen at the posterior aspect of the upper end of the tarso-metatarsus. These tendons become calcified in the adult fowl and are clearly visible in the lower part of Fig. 12.

The age at which the various changes take place in the developing hock will vary with such factors as breed, rate of growth and condition of housing. Our own work has been carried out with only a small number of birds of one breed and under only one set of conditions.

SUMMARY

The development of the normal hock joint of the fowl from the age of four weeks onwards has been followed by means of radiographs.

ACKNOWLEDGMENT

We are indebted to Mr. Gruber for the preparation of the illustrations.

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LEGENDS TO FIGURES

FIGS. 1 to 12.—Radiographs (4/5th natural size) of the developing hock joint of normal chicks. Left leg, except in Fig. 4.

FIG. 1.—Five-weeks-old chick, showing the three centres of ossification in the cartilage matrix, representing the tarsal bones.

FIGS. 2 AND 3.—The medial tarsal centre has begun to elongate and to approach the lateral tarsal; nine and 10 weeks respectively.

FIG. 4.—The upper tarsal centres are just in contact, but the union is not completely calcified. Fusion with the tibia has started; 10 weeks.

FIG. 5.—Fusion of the tarsal centres is nearly complete; ten weeks.

FIG. 6.—Fusion of the tarsal centres is complete. The epiphyseal gap is closing; eleven weeks.

FIG. 7.—The epiphyseal gap is closed. The distal tarsal centre extends the whole width of the joint; 14 weeks.

FIG. 8.—The tibiotarsus has been formed but the epiphyseal line can still be seen. The distal tarsal centre has become disc-shaped, and fusion with the metatarsus has started in the centre. The shadow of the developing sesamoid bone can be seen behind the medial condyle of the tibia and the outline of the hypotarsus is visible behind the metatarsus; 17 weeks.

FIG. 9.—The epiphyseal line of the tibiotarsus has almost disappeared. Fusion of the distal tarsal centre with the metatarsus is complete, but the epiphyseal line is clearly marked; 20 weeks.

FIG. 10.—Normal adult hock joint at one year. The triple origin of the tibiotarsus is no longer visible, but the epiphyseal line of the tarso-metatarsus can still be seen.

FIG. 11.—Normal adult hock joint at two years. The epiphyseal line has disappeared from the tarso-metatarsus.

FIG. 12.—Lateral view of the adult hock joint at 24 weeks, showing the sesamoid bone (appearing as a shadow in Figs. 8 and 9), and the hypotarsus with its longitudinal groove (seen as a shadow in Figs. 8-11). Calcification of the flexor tendons below the hypotarsus is just visible.

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OBSERVATIONS ON PEROSIS OCCURRING IN CHICKS USED FOR VITAMIN D_3 ASSAY

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INTRODUCTION

During routine assays of the vitamin D_3 content of various oils by the method of Olsson (1936) it was found that occasional chicks showed swelling and bending of the upper end of the metatarsus which made it difficult or impossible to measure the tarso-metatarsal distance in radiographs. These abnormalities were not related to the normal development of the hock (Bruce *et al.*, 1946) and were not rachitic in nature since they were found in birds receiving amounts of vitamin D_3 sufficient for complete protection from rickets. Radiographs of the hock were normally taken when the chicks were four to five weeks old, but a number of affected birds were kept for a further period and radiographs were taken at intervals. Symptoms typical of perosis developed and further work enabled us to study the earliest radiographic features of this condition, the macroscopic changes in the legs, and the histological changes in the affected bones. We have not, however, carried out any experimental work on the aetiology of the disease.

Perosis does not seem to be common in this country but cases occur among chicks reared intensively (Gordon, 1945) and it is possible that some are not recognised, particularly if actual slipping of the tendons fails to take place. Most of the work on perosis has been carried out in the United States where the disease causes serious losses among chicks and turkey poults reared intensively.

EXPERIMENTAL.

Housing and Care of the Birds.

All the birds used were males and were received at one-day-old from the Agricultural Research Council's Field Station, Compton. Until the end of the test period they were housed in electrically heated, all-metal battery brooders with $\frac{1}{2}$-inch mesh wire grid floors. For the first few days they were given a basal diet deficient in vitamin D; they were then wing-banded, weighed, divided into groups for test and the vitamin D supplements were added to the ration. At the end of the test period of three to four weeks, during which the birds were weighed weekly, the left hock joint was radiographed by extending the leg and placing it in contact with the film holder so as to obtain an antero-posterior radiograph.

Two types of basal diet were used. The first was similar to Diet No. 1 described in the B.S.I. Pamphlet No. 911 (British Standards Institution, 1940), with minor changes necessitated from time to time by war-time conditions. The second was similar to that described by Olsson (1941), and in this case the mineral supplement supplied as bone flour was withheld during the preparatory period. The composition of the diets was as follows :—

	B.S.I.	*Olsson (preparatory period)*
Whole yellow maize, ground ...	47	30
Whole wheat, ground	—	36
Bran	26	—
Dried skimmed milk	8	10
Defatted soya meal	—	13·5
Meat and bone meal	12	—
Dried yeast	—	3
Dried grass meal	5	6
Creta praeparata	0·5	—
Sodium chloride	0·5	1
Wood charcoal	—	0·5
	99	100
Oil being assayed	1	
	100	

	Olsson (test period)
Basal diet	94·9
Steamed bone flour	4·1
Oil being assayed	1
	100

The following figures from the analyses of the two diets are relevant to the problem of perosis :—

Diet	Protein	Ca	P	Ca/P	Mn
B.S.I. ...	16-18%	1·3-2·1%	1·0-1·3%	1·3-1·6	45-50 p.p.m.
Olsson (test) period)	18-19%	1·5%	1·0%	1·5	20-30 p.p.m.

INCIDENCE OF PEROSIS

The incidence of perosis recorded in the literature varies widely with the diet and method of housing and with the breed of fowl. It is well established that Rhode Island Red chicks are very susceptible to perosis and White Leghorns much less so. Perosis can usually be detected when the birds are four to seven weeks old, sometimes as early as three weeks, and further cases rarely develop after seven or eight weeks. The incidence recorded in chicks has varied from less than 1 per cent. to nearly 90 per cent., and in turkey poults Jukes (1940a) recorded a 100 per cent. incidence in birds on a diet deficient in choline.

The work of assaying cod liver oil was started in October, 1941, but perosis did not appear among the first 28 hatches. In April, 1943, two cases were recognised and from then until July, 1944, a few were found in each of 20 out of 22 hatches. The incidence during this period varied in different hatches from 0 to 3·9 per cent., a total of 107 cases being detected among 5,720 birds, of which 5,145 were Rhode Island Red – Light Sussex crosses, and the remainder Legbars. It must be emphasised that all the cases occurred during routine biological assay, and diagnosis of perosis has been based primarily on the appearance of the hock joint when radiographed at four to five weeks. At this age the disease could not usually be diagnosed from the external appearance of the hock alone, especially in birds with some degree of rickets caused by a low level of vitamin D, and so it was sometimes necessary to keep them for a further period before the diagnosis could be made with certainty. Although the incidence was low, it appeared to be affected by variations in the individual constituents of the diet.

The development of perosis seemed to be related to high body weight, two-thirds of the perotic birds being heavier than the average of their group. Affected birds did not seem to be handicapped as compared with normal birds unless the tendons of *both* legs were badly displaced, in which case the chicks were unable to stand and rapidly died from starvation.

Symptoms

The symptoms of perosis have been described by numerous workers: in the United States by Hunter and Funk (1930), Payne (1930) and Titus (1932); in Denmark by Nielsen (1942); and in Switzerland by Ebbell (1942).

Diagnosis of perosis in this work has been complicated by the fact that most of the birds were to some extent rachitic and the early signs of perosis—swollen hock joint, unsteady and ungainly gait, bandy or bow legs—may occur both in rickets and perosis. In our experience it is not possible to distinguish perosis from rickets from external appearances alone unless the tendons have slipped sufficiently from the tibial condyles to pull the leg outwards or inwards.

The results presented here do not give clear evidence of how soon the condition is manifested. One definite case of perosis was seen, however, in a two-day-old chick, in which both hock joints were swollen and the bird was unable to stand; dissection showed that the tendons in both legs had slipped over the outer condylar ridge. Another case was seen in an eight-day-old bird in which the tendons in the left leg were displaced. As a rule, however, perosis was not detected until the birds were four to six weeks old.

Typical abnormalities of stance are shown when perosis is fully developed (Figs. 1 to 6). The legs may be bowed with the feet turned in, or straddled with the feet turned out. One leg may be completely useless and turned out below the hock at an angle of 90° to the axis of the body. The hock joint is always swollen and is often flattened antero-posteriorly. Dissection has shown the tendon in a variety of positions—in the midline, on one condylar ridge, or outside the groove entirely.

RADIOGRAPHIC APPEARANCES.

Hall and King (1931) found no abnormality in radiographs of perotic chicks, apart from gross deformity of the tibia and metatarsus. Titus (1932) reported that the epiphyses were more densely calcified in perotic than in normal 12-weeks-old chicks. Gallup and Norris (1938) found the bones shorter and thicker in perosis but stated that calcification was normal: these findings were subsequently confirmed (Caskey, Gallup and Norris, 1939). Nielsen (1942), in an extensive study of the radiographic features in perosis, found that ossification of the tarsal centres and their fusion with the tibia and metatarsus are delayed in perosis, the bones become shorter and thicker, and closure of the epiphyseal lines at the lower end of the tibia and upper end of the metatarsus is delayed. Ebbell (1942) found no abnormality in radiographs of chicks with perosis and merely states that X-rays readily enable the disease to be differentiated from rickets.

The earliest change which we have observed in perosis is swelling of the upper end of the metatarsals and a more pronounced tapering towards their point of union. This change is not easily distinguished from rickets except in birds receiving high levels of vitamin D, in which the latter disease is unlikely to exist. Similar swelling of the lower end of the tibia is uncommon. When the tendons have slipped the abnormal stresses and strains to which the hock is subjected cause pronounced bending of the tibia close to its lower epiphysis. The first specific feature differentiating early perosis from rickets which we have noticed is bending of the swollen metatarsus just below its upper epiphysis (Figs. 7 and 8), the direction of bending being either anterior, lateral or medial. At this stage the tendons may slip from the condylar groove and come to rest on one of the condylar ridges. A bird in this condition is able to walk but all its

movements are clumsy. As the metatarsus increases in length the bend seems to travel distally down the shaft and is still recognisable some weeks later (Fig. 15). If the tendons slip completely from the condyles (Figs. 9 and 10) the metatarsus becomes rotated and the joint greatly swollen. A radiograph showing an anterior-posterior view of the metatarsus may then show a lateral view of the tibia (Fig. 14). Direct evidence that changes in the bones precede slipping of the tendon and are not a result of it is shown in two radiographs of the same joint (Figs. 8 and 9), the former being taken before the tendons slipped and the latter five days later when they had slipped to the outside of the hock.

Normally the birds were disposed of at four to five weeks, but a few perotic birds were kept and radiographed weekly. Union of the tarsals with the tibia and metatarsus did not seem delayed in perotic chicks but comparison with birds on a diet which failed to produce perosis would be necessary to decide this point.

MACROSCOPIC APPEARANCES OF THE HOCK.

Both legs from 23 birds with perosis were examined macroscopically, the ages of the birds varying from 26 to 47 days. In eleven birds there was no slipping of the tendons, in seven birds the tendons of both legs had slipped, in three birds the right and in two birds the left tendons only had slipped. In 16 cases slipping was to the lateral side of the hock, in the remaining three legs it was medial. That slipping of the tendon was not in any way related to the presence of rickets was shown by the observation that birds with slipped tendons on diets containing a high level of vitamin D showed no evidence of rickets in radiographs.

In the fowl the gastrocnemius tendon is thin and wide and lies superficially at the postero-lateral aspect of the hock. It is inserted into the hypotarsus and below this site the fibres merge with the podothecal sheath which encloses the flexor tendons. When " slipped tendon " occurs in perosis all the tendons running over the distal end of the tibia slip partially or completely to the lateral or medial side of the hock. They cannot slip from the upper end of the metatarsus since the gastrocnemius is firmly attached at this site and the flexor tendons are enclosed within the hypotarsal groove and canal and covered by the podothecal sheath. It will be seen that slipping of the tendons from the tibial condyles must inevitably cause rotation of the metatarsus in the direction in which slipping occurs. A leg in this condition can be completely extended only if the metatarsus is first brought into alignment with the tibia. The varying degree of flexion of the toes which occurs is due to pull on the flexor tendons caused by their displacement.

In almost all affected birds the metatarsus was bent and when the tendon had slipped it had done so towards the direction of the bend. In some birds the skin over the posterior surface of the

hock had been destroyed and the tibial condyles were exposed. In all affected birds the proximal end of the metatarsus was swollen. A longitudinal section through the hock joint of a normal (Fig. 16a) and a perotic (Fig. 16b) chick of the same age shows the characteristic changes in the metatarsus in perosis. The hock joint was always swollen and often contained an excess of fluid, sometimes bloodstained.

In birds on a diet low in vitamin D and with radiological evidence of rickets without evidence of perosis there was neither slipping of the tendon nor deformity of the leg bones.

This description is in agreement with the findings of others. Bending of the leg bones occurs in rickets only in very severe cases and then affects the whole length of the bones. Calcification is much reduced in rickets and the bones are tough but pliable: in perosis calcification is normal and the bones are brittle and cannot be bent.

HISTOLOGICAL STUDIES

Hall and King (1931) and Payne *et al.* (1932) failed to identify any significant histological changes in perosis: the latter concluded that the basis of the deformity was anatomical rather than histological. Hammond (1935)* found no change up to the 15th day in chicks of various ages given a diet which produced perosis and in chicks on a diet with which no perosis occurred. At 18 days, however, the cartilage columns in the zone of provisional calcification were shorter in perotic birds and osteoblasts were present around these columns in large numbers, sometimes forming a layer several cells deep. These observations were taken to indicate abnormally rapid ossification. Nielsen (1942) found that in perosis the essential change was reduced growth of the proliferative zone of cartilage at the lower end of the tibia and the upper end of the metatarsus. The epiphyseal line remained open longer in perotic chicks. The changes were contrasted with those occurring in rickets in which cartilage formation is normal but calcification is deficient.

Smith *et al.* (1944) have described changes in the bones of the forelegs of rabbits on diets deficient in manganese which resemble those occurring in perosis. The general picture suggested reduced osteogenesis and there was reduction in the number of spicules of cartilage in the zone of provisional calcification.

We have examined histologically the tibiotarsus and tarsometatarsus of 14 chicks, of which nine had perosis, two had perosis and rickets, one rickets alone, and two were normal birds on the same diet. The ages varied from 26 to 47 days. The bones were fixed in 10 per cent. formalin, decalcified in nitric acid, embedded in paraffin and stained with haematoxylin and eosin and with haematoxylin and picrofuchsin. It has not been possible to study the early

* We are greatly indebted to Dr. Hammond for kindly sending us a copy of his Thesis.

development of perosis in very young chicks or to compare the bones of perotic birds with those of birds from the same hatch reared on a diet which did not produce the condition.

The changes were more marked in the upper end of the metatarsus than in the lower end of the tibia and the former have been selected for illustration. A section through the proximal end of the metatarsus of a normal 31-day-old chick fed on the B.S.I. diet supplemented with sufficient vitamin D_3 to prevent rickets, is shown in Fig. 17. The three metatarsals are seen in longitudinal section and the centre of ossification representing the lower tarsal bone is seen above and separated from them by cartilage matrix. A comparable section through the metatarsus of a perotic chick of the same age and on the same diet, supplemented with an equal amount of vitamin D_3, is shown in Fig. 18. The lateral bending of the metatarsus just below its upper end is seen and it will be noticed that only the two outer metatarsals are visible, the middle one being excluded mainly because of the altered relative positions of these bones in perosis. The lower tarsal is seen embedded in cartilage matrix and its stage of development is similar to that in the normal bird. The medial metatarsal shows at its proximal edge regular tongues of ossifying cartilage similar to those seen in the normal bird. In the lateral metatarsal these tongues are shorter and more irregular. This change in the ossifying cartilage tongues is the only constant histological feature which we have found in perosis and it suggests an abnormality of the process of cartilage formation and replacement. In some perotic birds from which other leg bones were examined the same change was found. In some cases the columns of cartilage cells forming the proliferative layer of the epiphysis were irregular but we do not attach much importance to this observation because it was not a constant feature and because the irregularity of the columns may be in part an artefact due to bending of the upper end of the metatarsus causing the columns to be cut obliquely. Osteoblasts were numerous in both normal and perotic birds and no significant difference in their number or arrangement was detected.

A comparison of radiographs of the hock of the normal (Fig. 19) and of the perotic (Fig. 20) bird shows that calcification is not decreased in the latter.

There is thus little histological abnormality to account for the gross deformity occurring in perosis. The main feature seems to be a disorder of the process of cartilage growth and replacement. The occurrence of the shorter tongues of ossifying cartilage and their uneven arrangement may be an underlying cause of the bending of the metatarsus which we have shown to be an early change in perosis. Gross bending of the bones and slipping of the tendons is due secondarily to mechanical factors, such as high body weight, confinement, and wire floors.

Apart from its radiographic features, rickets is readily distinguished from perosis by the abundance of osteoid tissue, the paucity

of calcified bone, and the fibrous character of the bone marrow. Perotic chicks on diets low in vitamin D showed co-existent rickets and both conditions could be recognised in sections as well as in radiographs (Fig. 11).

DISCUSSION

Perosis was recorded in the literature as a form of rickets for some years before it was recognised as a specific condition. The first record we have found of the term perosis is in an article by Titus and Ginn (1931), and the use of this term instead of " deformed hock," " hock disease," " deforming leg weakness," "slipped tendon," etc., has done much to reduce confusion. Titus and Ginn found that perosis could be prevented by replacing wheat by 10 to 20 per cent. rice bran and also to some extent by adjusting the Ca/P ratio of the diet. From 1931 onwards a very large number of articles dealing with perosis has appeared, mostly from the United States where the disease causes considerable trouble in chicks and turkey poults. Perosis was produced when bone meal, Na or Ca phosphate, or various mineral mixtures were added to the basal diet. When other factors remained constant the incidence was higher in chicks reared in battery brooders, and in heavy breeds. An unsuitable Ca/P ratio was considered an important causative factor and the protective effect of rice bran was thought possibly due to an accessory food factor.

In 1936 Wilgus *et al.* found that whereas pure monocalcium phosphate increased the incidence of perosis, a crude technical grade of this substance afforded considerable protection which was due to the presence in it of Mn. The use of pure salts of Mn was soon found to be of value in preventing the disease. It now seemed that perosis was caused by deficiency of Mn, and was aggravated by excess of P. That this was not the whole picture, however, was shown by the fact that feeding high levels of Mn sometimes failed to protect and that autoclaving rice bran removed its perosis-preventing action. Jukes (1940a, 1940b) found that yeast partially protected against perosis and that choline chloride was the active agent. Subsequent work has confirmed the value of choline in preventing perosis, particularly in turkey poults. Nielsen (1942) was able to prevent perosis by adding 2 g. choline chloride and 0·2 g. Mn sulphate per kg. of food.

Deficiencies of Mn and choline seem to be the most important causes of perosis. The amounts required for prevention of the condition are: Mn, 0·003 to 0·01 per cent. (30-100 p.p.m.) according to the ration (Anon., 1944) and choline, 0·2 per cent. (Jukes, 1940b). Contributory factors are close confinement in battery brooders, wire floors, heavy breeds of birds and diets high in minerals.

We have not investigated the factors responsible for the cases of perosis encountered among the birds used for cod liver oil standardisation, but it is possible to consider which are the most likely. The Ca/P ratio and the levels of Ca and P in both diets are such that they are unlikely to produce perosis. The Mn content of the B.S.I. diet (45 to 50 p.p.m.) should be sufficient for protection but the Olsson diet may be deficient (25 to 30 p.p.m.). The choline content of the diets was not determined but the inclusion of 3 per cent. of dried yeast in the Olsson diet makes it improbable that choline deficiency was a causative factor. The heavy breed of birds used and the fact that they were kept throughout the experiment in battery

brooders with wire floors were probably contributory factors in causing the appearance of clinical perosis.*

SUMMARY

Perosis was found in 107 out of 5,720 chicks used for the assay of vitamin D_3.

An account is given of radiographic and histological studies of the early changes in the hock joint in perosis, together with a brief description of the symptoms and macroscopic features of the condition.

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* Since this paper was written we have found addition of 0·2 g. Mn sulphate per kg. food entirely prevented perosis in chicks given the Olsson diet, whereas addition of choline had no effect. It thus appears that perosis occurring with this diet may be due to Mn deficiency.

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LEGENDS TO FIGURES

FIGS. 1 to 6.—Photographs of young chicks with perosis.

FIG. 1.—Chick, six weeks old, showing moderate outward rotation of the right leg below the hock.

FIG. 2.—Chick, six weeks old, showing more marked outward rotation of the right metatarsus than in Fig. 1. Note the straight, widely spread toes of the normal leg.

FIG. 3.—Chick, seven weeks old, with the tendons slipped to the inside of both legs, giving a bow-legged stance.

FIG. 4.—Chick, seven weeks old, with perosis affecting both legs, but more severe in the left leg where the tendon has slipped to the inside of the hock. The toes are held abnormally. (Cf. the stance of the normal left leg in Fig. 2.)

FIG. 5.—Chick, eight weeks old, showing very severe perosis affecting both legs. In the left leg the tendons have slipped to the outside of the joint and the leg is held so far out from the body that it no longer touches the ground. The right leg is also severely affected as can be seen from the twisting of the toes, but the tendons have not slipped.

FIG. 6.—Chick, 14 weeks old, with perosis of both legs so severe that the bird was unable to stand without support. The swelling of the hock joints, the rotation of both metatarsi, and the twisting of the toes are clearly seen.

FIGS. 7 to 15.—Radiographs, 7/8th natural size, of abnormal hock joints.

FIG. 7.—Early perosis of left hock joint of a bird four weeks old. Note the swelling of the metatarsus and the apparent disappearance of the epiphyseal gap which this swelling causes in the radiograph. (Cf. normal hock shown in Fig. 19.)

FIG. 8.—Early perosis of left hock joint of a chick four weeks old. Note the bending as well as the swelling of the head of the metatarsus.

FIG. 9.—The same hock joint as in Fig. 8, five days later. The tendon has slipped causing rotation of the metatarsus and swelling of the tissues surrounding the joint.

FIG. 10.—Left hock joint of a chick four weeks old in which the tendons have slipped. (Cf. Figs. 7 and 8 in which the abnormalities of the metatarsus are not accompanied by slipping of the tendons.)

FIG. 11.—Left hock joint of a chick six weeks old, showing perosis as well as rickets. Note the enlarged epiphyseal gaps between the tibia and metatarsus typical of rickets and the swelling of the metatarsus typical of perosis. The deficient calcification of all the bones is due to rickets.

FIG. 12.—Typical severe rickets in the left hock joint of a chick five weeks old.

FIG. 13.—Apparently normal right hock joint of a bird 15 weeks old.

FIG. 14.—Left hock joint of the same bird as Fig. 13, showing bending of the tibia and rotation of the metatarsus due to perosis. Lateral view of tibia, anterior view of metatarsus.

FIG. 15.—Left hock joint of a bird nine weeks old showing bending of the metatarsus. The site of the original bend would have been in the position shown in Fig. 8 but growth in length has caused it to occupy a position further down the shaft.

FIG. 16.—Longitudinal section through the right hock of a normal chick (Fig. 16a) and a chick with perosis (Fig. 16b), both 31 days old and on levels of vitamin D_3 sufficiently high to prevent rickets. The metatarsus of the perotic leg is swollen and bent, whereas the lower end of the tibia seems unaffected. The lower tarsal centre occupies a more posterior position over the metatarsus because of bending of the upper end of the latter bone. This bending anteriorly occurs in addition to the lateral bending described in the text and illustrated in Figs. 8, 15, 18 and 20.

FIGS. 17 to 20.—Photomicrographs and radiographs of the hock of a normal and a perotic chick, both 31 days old and on the same level of vitamin D_3 (adequate to prevent rickets).

FIG. 17.—Section through proximal extremity of metatarsus, with the lower tarsal centre situated above it. Normal bird. Haematoxylin and picrofuchsin. ×7.

FIG. 18.—Similar section to that shown in Fig. 17, but from a bird with perosis. Note lateral bending of the metatarsus and irregular tongues of ossifying cartilage in the lateral metatarsal (left of figure). More detailed description in the text. Haematoxylin and picrofuchsin. × 7.

FIGS. 19 AND 20.—Radiographs of the hocks from which Figs. 17 and 18, respectively, were made. The plane shown in the radiographs is the same as that of the sections.

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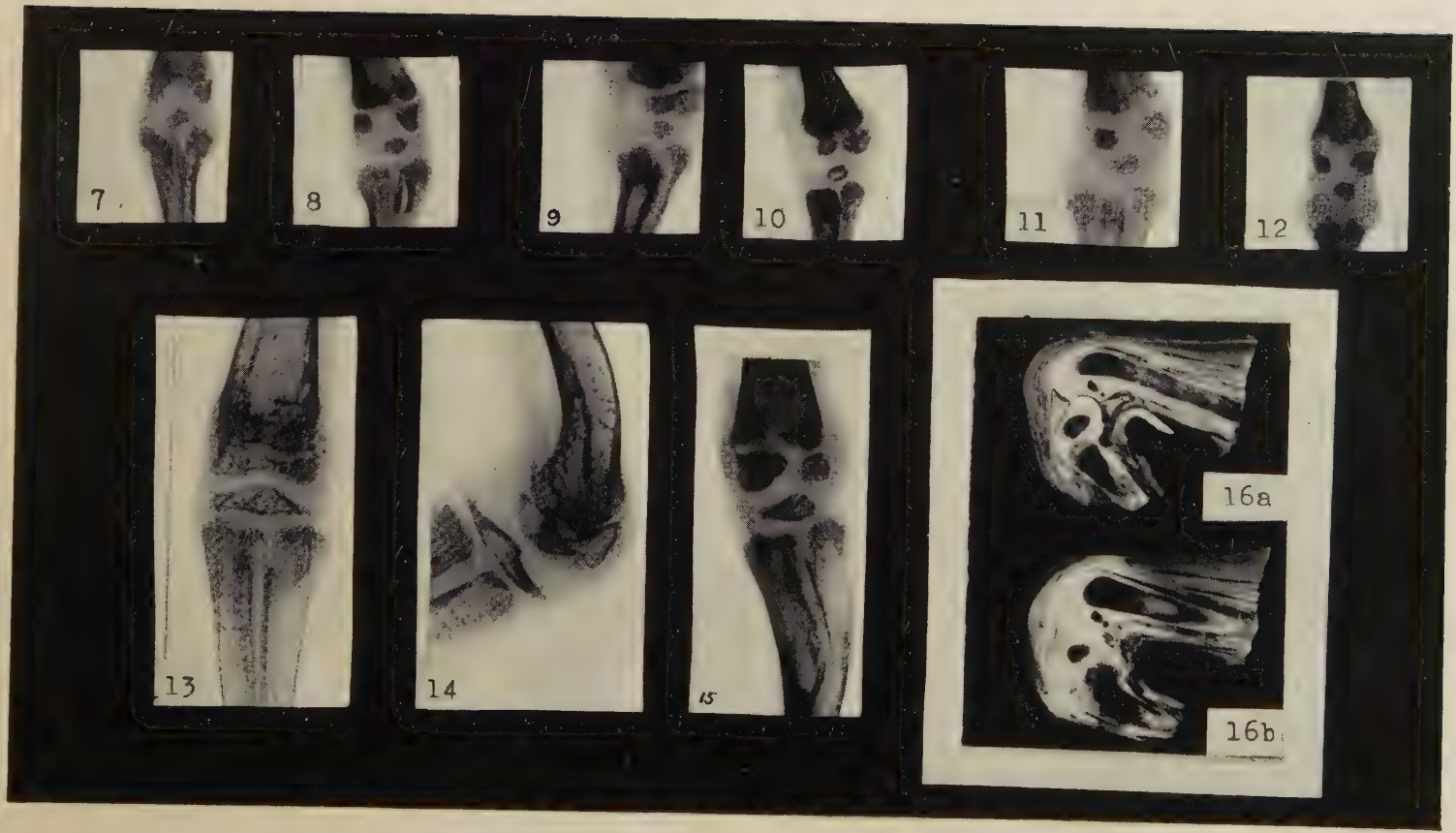
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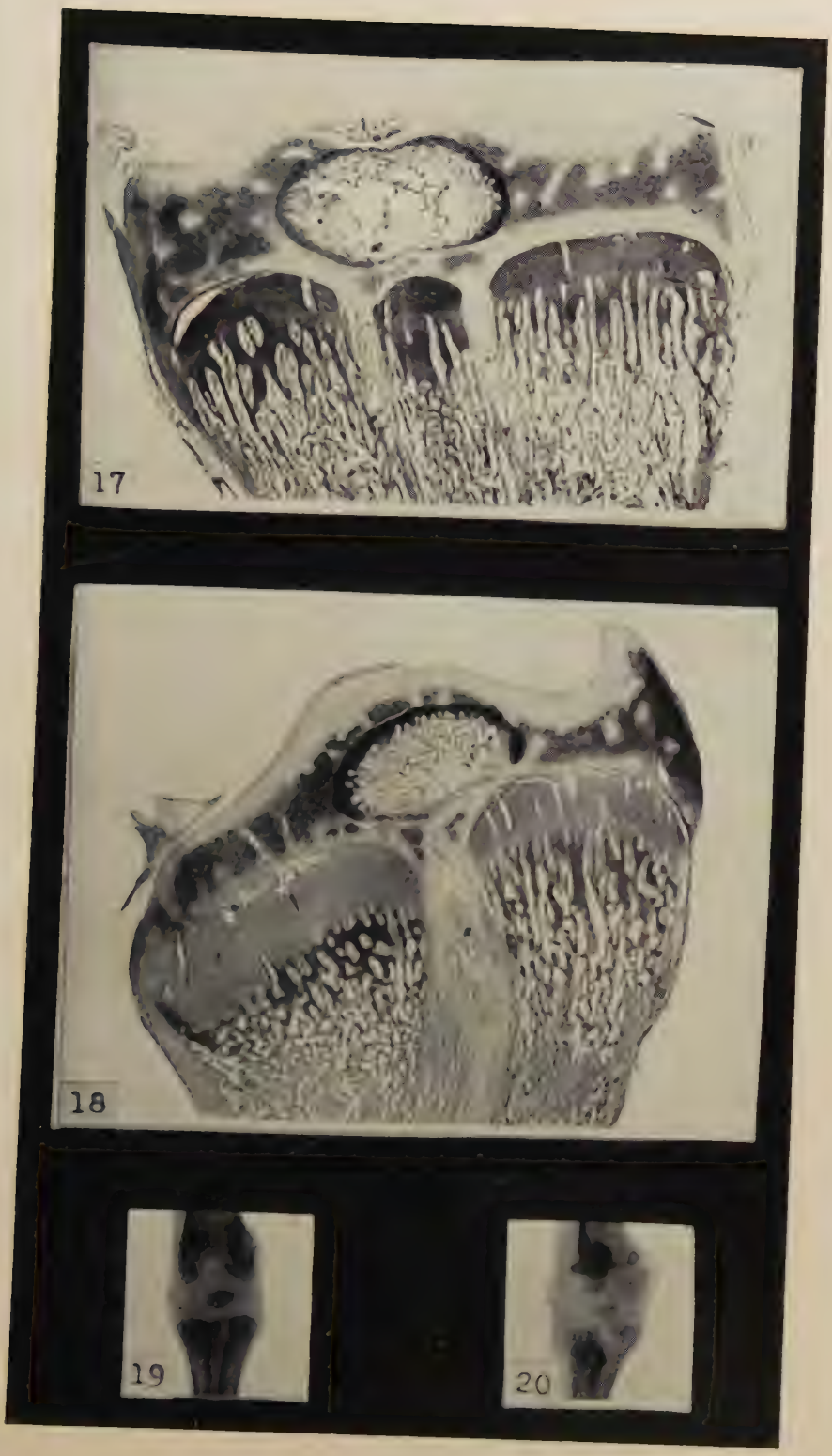
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OBSERVATIONS ON A POSSIBLE CASE OF JAAGZIEKTE IN A SHEEP IN PALESTINE

By

I. H. Pattison,

Government Veterinary Laboratory, Tel-Aviv, Palestine.

INTRODUCTION

In April, 1944, part of one lung, a kidney, and part of the small intestine of a sheep from an agricultural settlement in the plain of Esdraelon were received in the laboratory. By mouse inoculation and toxin-antitoxin tests, the cause of death was determined to have been enterotoxaemia due to *Cl. welchii*, Type D. Examination of the piece of lung, however, revealed the presence of a greyish nodule, irregular in shape but immediately under the pleura measuring some 4 cm. across. There was no evidence of pus formation or caseation, and a small amount of froth could be expressed by squeezing. Histological preparations revealed an unusual picture suggesting a fibro-adenoma. The possibility that this was a jaagziekte lesion was entertained, and a more detailed histological study was undertaken because hitherto the presence of jaagziekte in Palestine had not been suspected.

METHOD OF EXAMINATION

The tissue was fixed in 10 per cent. formol-saline, dehydrated in alcohols of increasing strength, cleared in chloroform, and embedded in paraffin. Eleven blocks were made from different parts of the nodule, and sections were stained with haematoxylin and eosin, with Heidenhain's iron haematoxylin, and with van Gieson's stain.

HISTOLOGICAL FINDINGS

The following changes were not all noted in any single section, and the description is a composite one based on examination of sections from all parts of the nodule.

Under a low magnification the tissue had a markedly glandular appearance, reminiscent of healthy mammary gland, and in places the normal lung structure was entirely obliterated. In other parts islands of abnormal tissue were surrounded by more or less normal lung.

A higher magnification revealed that in those places where the alveolar structure of the lung was still obvious the interalveolar tissue was thickened and contained an abnormal accumulation of lymphocytes, monocytes and occasional eosinophils; a few polymorphs were noted also, but appeared to be lying within small blood vessels. In many places there was a marked increase of fibrous tissue in the alveolar walls.

The alveolus, in general, showed profound abnormality and was lined by epithelium that was cubical, or columnar, or that showed proliferation to a remarkable degree (Fig. 1). Some alveoli, under high magnification, resembled a gland duct in cross section. The alveolar epithelial cells stained darkly (so that attention was immediately focused on them during examination under low magnification), and their large nuclei were very distinct; occasionally these lining cells appeared to contain two nuclei. In some alveoli proliferation of the lining cells was in ring form, but in others only part of the epithelium was proliferated and protruded into the lumen of the alveolus as a papilliform outgrowth (Fig. 2).

The majority of bronchioles and small bronchi showed the same type of proliferation of the lining epithelium as was present in the lining cells of the alveoli. Some of the proliferated cells appeared to contain two nuclei. Cilia were obvious on the surface layer of proliferated cells in some small bronchi. Aggregations of lymphocytes were associated with many bronchioles and small bronchi, and occasional groups of these cells apparently unrelated to bronchiole or bronchus, were noted.

Here and there throughout the tissue were clusters of large, rounded or oval cells the cytoplasm of which stained pink with eosin. These cells had either one or two nuclei, oval or irregular, which were usually eccentrically placed; the cytoplasm was frequently vacuolated. In many places these cells had the appearance of being stuck together, but elsewhere the complete outline of each cell was clearly visible (Fig. 3).

Worm parasites were found in many, but not all, sections. In some parts the histological picture was almost that of ordinary verminous pneumonia. There was considerable development of fibrous tissue in the region of the parasites, and occasional giant cells of foreign body type, apparently related to parasites, were noted.

Myxomatous tissue.—As has been mentioned above, the interalveolar tissue showed generalised thickening and cell infiltration, but in many places collections of cells were noted so arranged, in sweeps and whorls, as to form structures apparently distinct from the rest of the lung. In most cases these collections of cells were quite sharply demarcated from the surrounding lung tissue, and constituted, apparently, aggregations of myxomatous tissue (Fig. 4). In some cases the amount of stroma in this myxomatous tissue was great and the cells scanty; in others the cells were much more numerous. In general, this tissue did not appear to be related to worm parasites, but in one section one myxomatous mass, well developed, contained worm parasites and numerous giant cells in its centre.

Slight oedema was noted in places, but to no greater extent than has sometimes been observed in sections of the lungs of sheep dying of other diseases. One very small purulent focus consisting of polymorphs and necrotic tissue was seen. The pleura was thickened.

DISCUSSION

The histological picture given above resembles very closely descriptions of the histological findings in cases of jaagziekte in South Africa (*e.g.*, de Kock, 1929), and is also very similar to the histological findings described in the disease of sheep in Iceland called " epizootic adenomatosis " (Dungal et al., 1938). It is now considered (Taylor, 1937 ; M'Fadyean, 1938) that jaagziekte occurs in Great Britain ; moreover, histological descriptions from that country (*e.g.*, Blakemore and Bosworth, 1941) agree very closely with the findings reported in this paper. M'Fadyean (1938) has given it as his opinion that jaagziekte in South Africa, " epizootic adenomatosis " in Iceland, and the " verminous pneumonia " reported by himself in Great Britain in 1894, are the same disease, and that the " progressive pneumonia " of sheep in Montana, described by Cowdry and Marsh (1927) is probably of the same nature.

Until the case here reported was encountered there was no suspicion that jaagziekte of sheep occurred in Palestine. It is important, therefore, to consider how far the information now available affords evidence that the disease is in fact present in this country. From the clinical point of view it must be stated at once that at the time of writing there is no definite evidence to prove the existence of this disease. No veterinary surgeon to whom the writer has spoken has been able to give any information indicating that the disease has been observed by him, and, as far as is known, no clinical symptoms of jaagziekte were observed in the animal from which the material described in this paper was obtained. Death was due to enterotoxaemia, and the discovery of the nodule in the lung was purely accidental.

It might be argued that such complete lack of clinical evidence of the presence of jaagziekte in Palestine suggests that this was not a case of jaagziekte, or that, if it was, it represents an isolated example of a rare condition. As is probably true in most countries, not by any means all the sheep that die in Palestine are examined *post-mortem* by a veterinary surgeon, and of those so examined only a small number is examined in circumstances that make histological investigations possible. During the years 1943 and 1944 some 248 specimens from sheep were received in this laboratory, but in only some 25 cases were the lungs included. Naturally, the diseases of known economic importance, *e.g.*, anthrax, enterotoxaemia, are those uppermost in the mind of a veterinary surgeon submitting material for laboratory examination. It is certain, therefore, that numerous single cases of death from one cause or another must occur without causing alarm and without making a laboratory examination imperative. Blakemore and Bosworth (1941), working in Great Britain, appear to have encountered the same difficulty in reconciling the existence of a disease with such typical histological findings as those of jaagziekte, with the dearth of clinical reports concerning it ; they

say, " The evidence brought forward by M'Fadyean would indicate that the disease has existed here for many years but the symptoms and lesions are so striking that it is difficult to explain the absence of clinical reports relating to its occurrence." The present complete lack of clinical evidence of the presence of jaagziekte in Palestine, therefore, does not necessarily mean that this was not a case of the disease, or if it was such a case, that the disease is a rarity.

It has next to be decided to what extent a diagnosis can be based entirely on histological findings. M'Fadyean (1938) has said of jaagziekte, " The lesions are pathognomonic both in respect of their appearance and their histology," and such a statement from a veterinary pathologist of great experience is strong presumptive evidence that any condition other than jaagziekte showing a histological picture such as that described in this paper must be very rare indeed. Histological preparations from this case were sent for examination to Dr. E. L. Taylor, of the Ministry of Agriculture and Fisheries Veterinary Laboratory, England, and he has been kind enough to allow publication of a quotation from a letter, as follows, " I . . . have little doubt that your suspicions are correct, and that the lung was from a sheep suffering from jaagziekte." However, Dr. Taylor lays stress on the importance of considering the clinical picture as well as the histopathology, and it is most unfortunate that no clinical evidence can be produced to support the histological findings. In the writer's opinion, however, the histological picture is apparently the same as that reported by other observers from known cases of jaagziekte.

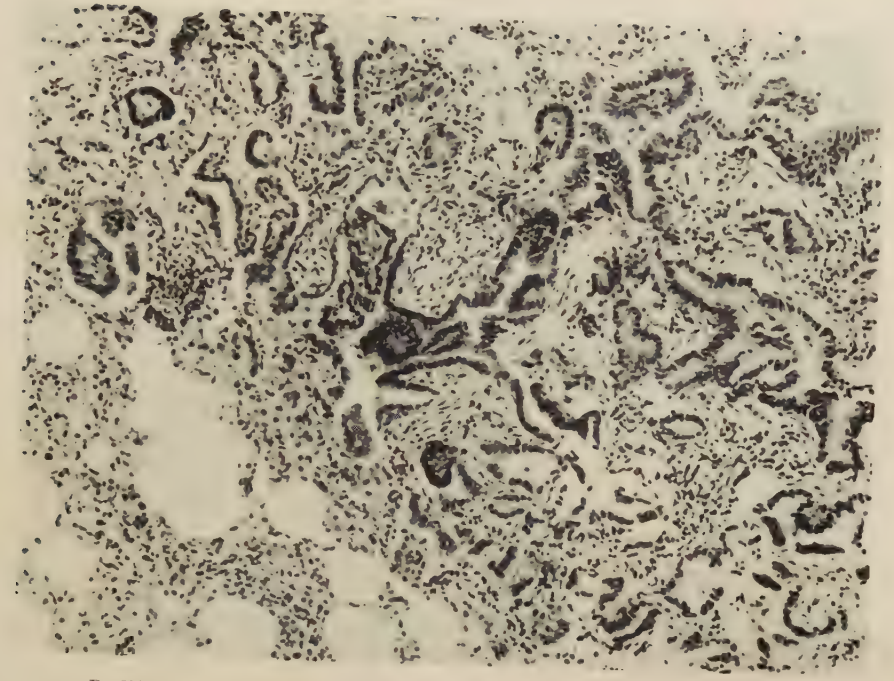
In the absence, therefore, of indisputable evidence that this was a case of jaagziekte it becomes necessary to consider if any other explanation of the condition is consistent with the findings. The only possibility appears to be that it was a tumour. In the writer's opinion, however, it was not a tumour, although the close resemblance to a neoplastic process is undeniable. Bonne (1939) has drawn attention to the morphological similarity between jaagziekte in sheep and certain cases of cancer of the lung in man. He describes similar changes in the lungs of guinea-pigs and mice, and says, " There exists apparently a group of morphologically closely related diseases producing in the lungs of several species of animals, *e.g.*, sheep, guinea-pigs and mice, as well as of man, a diffuse new growth of alveolar and bronchiolar cells." The writer believes that the case described here could be included in this generalisation.

No incontrovertible evidence, therefore, can be advanced either to prove or to disprove that this was a case of jaagziekte, but the balance of evidence is in favour of its being an example of that disease.

SUMMARY

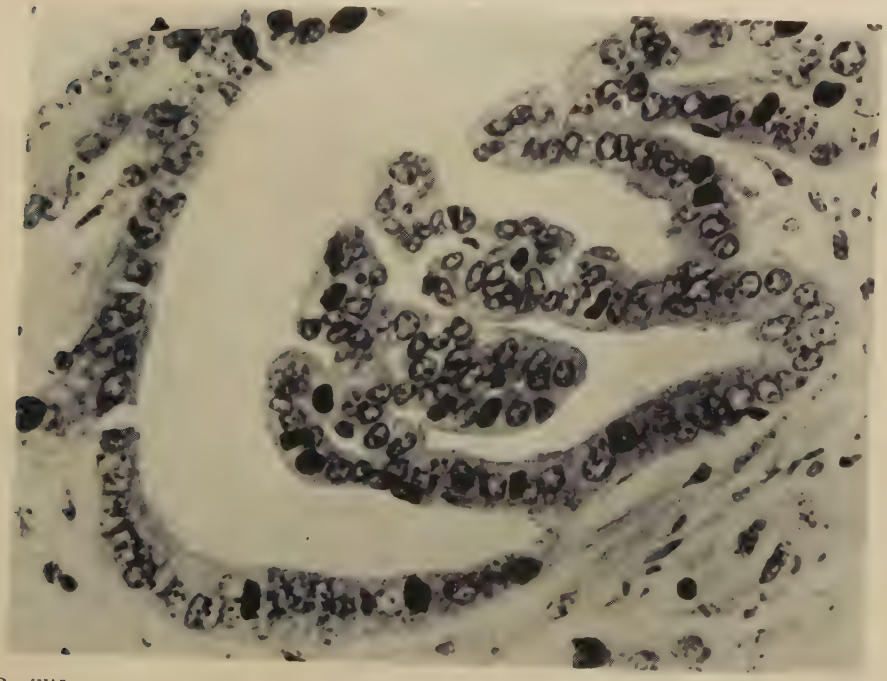
The histological structure of a nodule found in the lung of a sheep that died of enterotoxaemia is described, and the possibility that this was a subclinical case of jaagziekte is discussed.

Fig. 1.



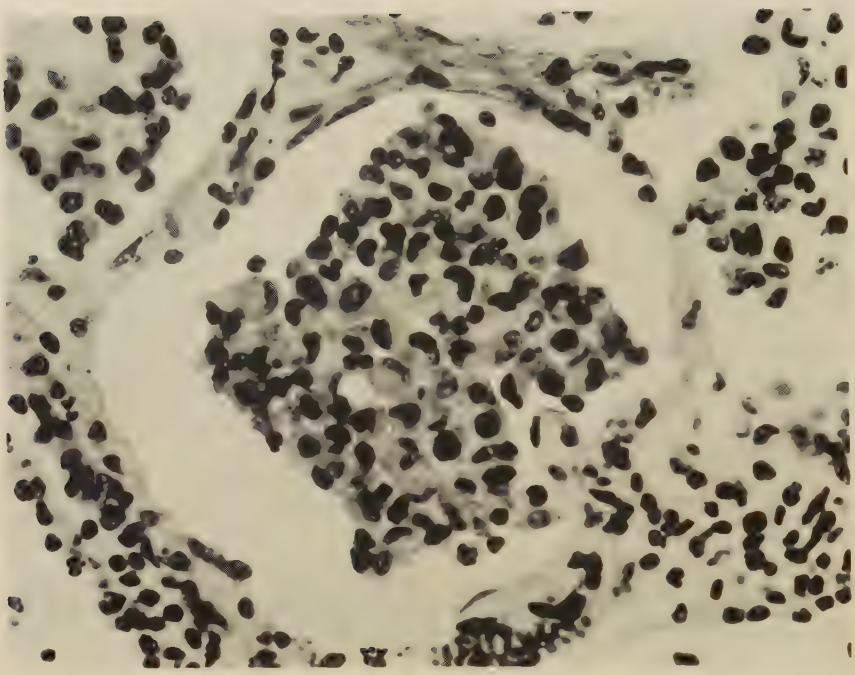
Proliferating epithelium. (Heidenhain's iron haematoxylin. × 420.)

Fig. 2.



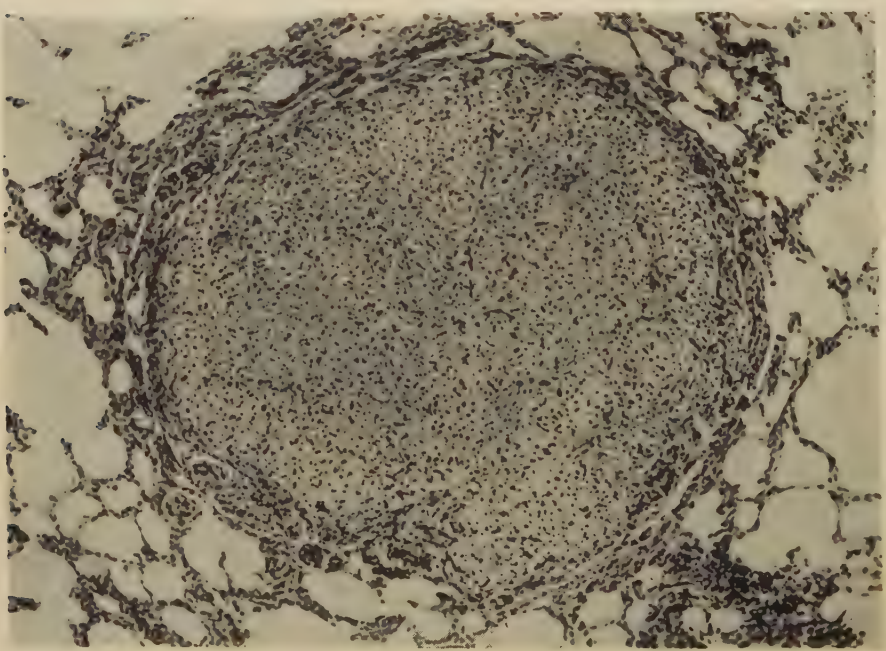
Papilliform outgrowth of proliferating alveolar epithelium. (Heidenhain's iron haematoxylin. × 520.)

Fig. 3.



A mass of large, rounded or oval cells lying within an alveolus. (Heidenhain's iron haematoxylin. × 520.)

Fig. 4



A mass of myxomatous tissue sharply demarcated from the surrounding lung tissue. (Heidenhain's iron haematoxylin. × 110.)

ACKNOWLEDGMENTS

I am most grateful to Dr. E. L. Taylor, of the Ministry of Agriculture and Fisheries Veterinary Laboratory, Weybridge, England, for his interest in this case, for examining histological preparations sent to him, and for reading this paper, and to Dr. L. Doljanski, Department of Experimental Pathology, Hebrew University, Jerusalem, for time and patience willingly spent in preparing the photomicrographs.

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* *Meyer, E., and Arnold, I., Amer. J. Digest. Dis., 5:418*

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